DEVELOPMENT AND USE OF AN *IN VITRO* SYSTEM TO STUDY THE RIPENING PHYSIOLOGY OF STRAWBERRY FRUIT

By

PENELOPE M. PERKINS-VEAZIE

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1988

TO MY HUSBAND, RAYMOND

ACKNOWLEDGMENTS

Drs. D.J. Huber, J.K. Brecht, Earl Albregts, C.B. Hall and T.E. Humphreys have been an excellent and most helpful committee. I am most appreciative of the guidance given by Drs. D.J. Huber and J.K. Brecht, whose perception and expertise have been exemplary. Their prompt editing of my manuscripts has been a delightful experience.

The teamwork displayed by the staff and faculty in postharvest research have taught me invaluable and unforgettable lessons. They have always been willing to provide suggestions, answer questions and help explain experimental techniques.

I also thank the staff and faculty at the Dover Agricultural Education and Research Center, who planted and maintained much of the plant material used in these experiments. These people have exemplified good field production practices and teamwork.

Special thanks are given to Dr. Chris Chase, whose loan of the green chair provided a comfortable seat for the many hours of contemplation of literature and research.

The help provided by Dean Jack Fry over the past few years is most deeply appreciated.

TABLE OF CONTENTS

		Page
ACKNOWLEDGMENTS		iii
ABSTRACT		ix
CHAPTERS		
I	INTRODUCTION	1
II	LITERATURE REVIEW	4
	Strawberry Fruit Growth and Ripening	4
	Strawberry Fruit Compositional Characteristics During Ripening	11
	Relationship of Respiration and Ethylene to Fruit Ripening	17
	Ethylene Synthesis	26
	Summary	34
III	DEVELOPMENT AND EVALUATION OF AN IN VITRO SYSTEM TO STUDY DETACHED STRAWBERRY FRUIT RIPENING	37
	Materials and Methods	39
	Results	43
	Discussion	55
	Conclusions	59
IV	RESPIRATION AND ETHYLENE PRODUCTION IN DEVELOPING STRAWBERRY FRUIT GROWN IN VITRO	61
	Materials and Methods	63
	Results	67
	Discussion	79
	Conclusions	83
V	REGULATION OF ETHYLENE PRODUCTION IN STRAWBERRY FRUIT	85
	Materials and Methods	87
	Results	91
	Discussion	98
	Conclusions	100

Page

VI SUMMARY AND CONCLUSIONS..... 101

LIST OF REFERENCES..... 104

BIOGRAPHICAL SKETCH..... 118

LIST OF TABLES

Table		Page
3-1	Relationship between the color of *in vitro* grown strawberry fruit and HunterColorlab L,a,b values....	42
3-2	Effect of age on *in vitro* growth of 'Pajaro' strawberries harvested and placed in solutions at 6, 12, or 18 days after anthesis.....	49
3-3	Rates of growth *in vitro* for primary, secondary or tertiary fruit order.....	52
3-4	Comparison of *in vitro* fruit weight gain and ripening to field fruit.....	53
3-5	Comparison of length and diameter of *in vitro* and field-ripened fruit of the same anthesis date...	54
5-1	Comparison of ACC content, EFE activity and ethylene production from strawberry fruit harvested from the field at different color stages..	92
5-2	Effect of STS and ACC addition to vase solutions with strawberry fruit on ethylene biosynthesis of fruit grown *in vitro*.....	94
5-3	Comparison of ACC content, EFE activity and ethylene production from green strawberry fruit receptacle tissue and calyx tissue after incubation in 1 mM ACC in vase solutions for 2 days.....	96

LIST OF FIGURES

Figure		Page
3-1	Effect of cultivar and sucrose on cumulative fruit growth *in vitro*	44
3-2	Effect of age and sucrose on cumulative growth of 'Pajaro' fruit *in vitro*	46
3-3	Effect of fruit age at harvest on cumulative growth *in vitro*	48
3-4	Effect of fruit order on cumulative growth *in vitro*	51
4-1	Changes in respiration and ethylene in strawberry fruit harvested at selected stages of development	64
4-2	Influence of propylene on respiration (A) and weight gain (B) of strawberry fruit harvested 20 days postanthesis (white)	69
4-3	Effect of propylene on respiration (A) and weight gain (B) of fruit harvested 12 days postanthesis (green)	70
4-4	Ethylene production (A), respiration (B) and weight gain (C) of fruit harvested at 20 days postanthesis (white) and held in vase solutions with 0 or 1 mM ACC	72
4-5	The effect of ACC on ethylene production (A), respiration (B) and weight gain (C) of fruit harvested at 14 days post-anthesis (green) stage of development and placed in vase solutions	74

4-6 Ethylene production of fruit harvested at 14 days post anthesis (green) and placed in vase solutions containing 0, 1, or 5 mM ACC..... 75

4-7 Respiration (A) and weight gain (B) of fruit harvested at 14 days post anthesis (green) and placed in vase solutions with 0, 1, or 5 mM ACC..... 77

4-8 The effects of ACC and silver on ethylene production (A), weight gain and color (B) of fruit harvested at 14 days post anthesis (green) and placed in vase solutions with 0 or 1 mM ACC and/or STS..... 78

5-1 Ethylene production from calyxes and whole fruit harvested at the green stage after calyx removal and treatment with 1 mM ACC..... 97

Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

DEVELOPMENT AND USE OF AN *IN VITRO* SYSTEM TO STUDY THE RIPENING PHYSIOLOGY OF STRAWBERRY FRUIT

By

Penelope M. Perkins-Veazie

April 1988

Chairman: Donald J. Huber
Major Department: Horticultural Science (Vegetable Crops)

Attempts to study and modify strawberry (*Fragaria* X *ananassa*) fruit ripening have been limited by the continued growth of this fruit during ripening and the failure of the strawberry to continue to ripen once detached. The purpose of this work was to develop a system which would permit normal ripening of detached, immature strawberry fruit under controlled conditions. This system was then implemented to study the physiological changes involved in ripening.

Normal ripening of detached fruit was achieved by placing their peduncles in a solution of 200 ul.liter^{-1} hydroxyquinoline sulfate plus 88 mM sucrose. 'Pajaro' strawberry fruit harvested at 50% maturity and placed

in vitro consistently gained more weight than did fruit harvested at 25% or 70% maturity. Fruit placed *in vitro* gained less cumulative weight than those in the field, but both *in vitro* and field-grown fruit ripened simultaneously.

Ethylene production and respiration trends of fruit harvested from the field at different stages of color development were characteristically nonclimacteric. However, fruit maintained in a 5000 $ul.liter^{-1}$ propylene atmosphere or inclusion of 1 mM 1-aminocyclopropane-1-carboxylic acid (ACC) in vase solutions failed to elicit an elevated respiration rate, although weight gain and color development were accelerated. Addition of 0.5 mM silver thiosulfate to vase solutions suppressed fruit growth but did not delay ripening.

The ACC content of strawberry fruit changed little throughout ripening, while ethylene-forming enzyme (EFE) activity and ethylene production decreased as fruit developed from the green to the white stage. Although no ACC synthase could be detected in strawberry fruit at any stage of maturation, the increased levels of ACC found in silver-treated fruit provided evidence for the presence of this enzyme. Inclusion of ACC in vase solutions increased receptacle ACC content, stimulated EFE activity and greatly increased ethylene production. Both EFE and ACC synthase activities may control ethylene biosynthesis in strawberry fruit, but active ACC synthase must be isolated from this fruit before the mechanism(s) responsible for low ethylene production can be ascertained.

CHAPTER I
INTRODUCTION

Fruits are generally classified into two categories, depending on their pattern of ripening. Climacteric fruit such as bananas, avocados, apples and tomatoes exhibit elevated respiration and ethylene production during ripening. Other fruit types, such as grapes, watermelons and strawberries, are nonclimacteric. As these fruit mature and ripen, there is a downward drift in respiration rate and no increase in ethylene production. Climacteric fruit have been intensively studied in attempts to establish a link between ethylene and other ripening parameters. Limited work has been conducted with nonclimacteric fruit due to the inability of these fruit to continue normal ripening once detached, and the lack of ripening markers such as polygalacturonase activity or increased ethylene production as found in climacteric fruit.

Research on strawberry fruit has been extensive but information on their ripening processes is lacking. The roles and types of growth regulators in strawberry fruit have been intensively studied, including changes in abscisic acid (ABA) and auxin in achenes and receptacles and the action of auxin in assimilate transport. As yet, the actual

involvement of these hormones in the ripening of all fruit types is unknown. A lack of response to applied ethylene has been demonstrated in strawberries and the role of endogenous ethylene is speculative. Sucrose is the major assimilate transported to the fruit during growth, yet its fate in the fruit is unknown. Cell wall changes contribute to strawberry fruit softening but the process is not triggered by polygalacturonase.

Strawberries have several unique features compared to other fruit. The true seeds (achenes) are located on the outer surface of the fruit. This morphological characteristic resulted in the first identification of the involvement of auxin from seeds in fruit growth and development. In addition, strawberries have an extended peduncle joining the receptacle to the parent plant. The presence of this extended peduncle suggests the possibility of using floricultural techniques to study ripening of detached strawberry fruit.

Strawberry fruit ripening is difficult to study on detached fruits. Strawberries exhibit a high respiration rate and continued growth through ripening. When detached at a preripe stage, the fruit quickly become spongy and exhibit only limited increases in anthocyanin and soluble solids contents. Most of the previous studies with strawberries were done with attached fruit. Using attached fruit for studies adds a number of potential cultural variables, which have to be identified, controlled and evaluated in an analysis of fruit ripening.

Since strawberry fruit fail to continue normal development once detached, the first objective of this work was to develop a system which would permit normal ripening of detached, immature strawberry fruit. Once this system was developed and optimized, the influence of ethylene on ripening changes was monitored. Finally, ethylene biosynthesis enzymes in strawberry fruit were measured and the relationship of this pathway to fruit ripening was determined, in the hope that the role of ethylene in strawberry fruit ripening could be elucidated.

CHAPTER II
LITERATURE REVIEW

The strawberry (*Fragaria* X *ananassa*) fruit has a relatively short life span of 30 to 50 days, depending on environmental conditions and genotype (Dana, 1980). As the fruit ripens, its appearance changes dramatically, yet few of the physiological changes associated with ripening have been examined in detail. Historically, strawberry fruit have been used more extensively in studies of parthenocarpy and auxin action (Coombe, 1976) than in ripening studies.

Strawberry Fruit Growth and Ripening

Fruit Morphology

The strawberry is an aggregate fruit. A number of ovaries belonging to a single flower adhere as a unit on a common receptacle (Shoemaker, 1978). Fruitlets, or achenes, are attached to the outer surface of the swollen receptacle in spirally arranged rows (Abbott et al., 1970). The achenes are connected to the receptacle by vascular strands and the epidermal layer of the receptacle (Avigdori-Avidov, 1986). The receptacle with achenes is generally referred to as the fruit.

The receptacle organ consists of pith tissue, forming a central cylinder, which is surrounded by cortical tissue containing parenchymal and epidermal cells (Havis, 1943). Vascular bundles traverse the pith and cortex to the achenes on the outer surface of the cortex and act as extensions of the peduncle from the vascular tissue (Lis and Antoszewski, 1982).

Each fruit is attached to the parent plant by a relatively long peduncle. Each fruit is initiated from a growth axis terminating in an inflorescence (Dana, 1980). A primary (1^{o}) fruit is initiated at the terminus of the inflorescence, while secondary (2^{o}) and tertiary (3^{o}) fruit are initated from primordia located between bracts on the inflorescence below the primary bloom.

Fruit Growth

The growth of the strawberry fruit, measured by changes in fresh weight, dry weight, fruit length or diameter, generally is reported to be single sigmoidal (Crane and Baker, 1953). Single-sigmoidal growth is characterized by an initially slow growth rate followed by a phase of exponential growth rate, then a declining growth (Bollard, 1970). However, unlike other fruits classified as single sigmoidal, such as muskmelon (Pratt et al., 1977) or apple (Biale and Young, 1981), strawberries ripen prior to the cessation of growth (Smith and Heinze, 1958).

There is some evidence which indicates that strawberry fruit growth may be other than single sigmoidal. Some

researchers have demonstrated double-sigmoidal growth, depending on cultivar and measurement intervals during development. Archbold and Dennis (1984) measured fruit of short-day plants at 3-day intervals throughout development and reported a double-sigmoidal growth pattern. Stutte and Darnell (1987) reported single sigmoidal growth for a day-neutral cultivar. Another day-neutral cultivar was reported to exhibit apparent single sigmoidicity when measured every 6 days (Mudge et al., 1981) and, in a later study, double sigmoidicity when measured every 3 days (Veluthambi and Pooviah, 1984).

Achene growth. Thompson (1964, 1969) studied both achene and receptacle growth of strawberry fruit and concluded that achene development occurs prior to receptacle enlargement. Upon maturation of the embryo, receptacle growth becomes rapid and ripening is initiated. The achenes consist of embryo, endosperm, nucellus and carpel tissue (Thompson, 1963). The endosperm is a free-nuclear layer bounding the embryo sac until 10 to 14 days after anthesis, after which time the endosperm becomes a cellular layer. In fertilized ovules, embryo formation is completed 10 days after anthesis. The vascular bundle of each achene supplies nutrients to the achene and surrounding parenchyma cells (Antoszewski, 1973). Studies with radiolabelled auxin have shown that auxin is translocated basipetally through the vascular bundles from the achenes to the peduncle, whereas nutrients move acropetally (Antoszewski, 1973).

Receptacle growth. Receptacle development is generally expressed in terms of changes in color and size. Stages of development of the receptacle are usually classified as small green, large green, white, pink or red (ripe), (Huber, 1984; Culpepper et al., 1935). Fruit are at their maximum size at the red stage (Huber, 1984). Fruit reach the white stage approximately 21 days postanthesis and are fully red (ripe) within 30 to 40 days (Dennis et al., 1970). Time to fruit ripeness is strongly dependent on cultivar and environmental conditions, particularly temperature (Dana, 1980).

As with other fleshy fruits, most of the size increase in strawberry fruit is due to cell expansion. Havis (1943) measured cell volume in transverse sections of fruit harvested from the time of pollination through fruit ripeness. He concluded that cell enlargement accounted for 90% of postanthesis fruit growth. Knee et al. (1977) reported that cell division ceased in the receptacle tissue at 7 days after petal fall. Fresh weight increased rapidly between 14 and 28 days after petal fall and then plateaued between 28 and 35 days, concomitant with fruit reddening.

Factors in the fruit effecting growth. It has long been observed that primary fruit are larger than secondary or tertiary fruit. Sherman and Janick (1966) calculated that 3^{o} and 2^{o} fruit attained 25% and 50% fresh weight, respectively, of the size of the 1^{o} fruit. These size

differences are believed to be the result of the greater number of achenes on primary fruit (Abbott et al., 1970). Webb et al. (1978) observed that a minimum number of achenes was necessary for fruit development. They speculated that the number of achenes was set at flower initiation, with berry size determined at the flower primordia stage. Although the relationship between the number of achenes and berry weight is linear, the degree of receptacle tissue sensitivity to hormones secreted from the achenes also may be involved (Moore et al., 1970). Large-fruited clones had a greater relative difference in size between primary and secondary fruit than did small-fruited cultivars. Webb et al. (1978) found that the maximum area of receptacle tissue influenced by achenes was limited to 0.165 cm^2. Lis and Antoszewski (1982) reported that the response of berries without achenes to exogenously applied auxin was greater in primary than in secondary fruit. This difference may be related to other factors, such as vascular bundle size, or the potential for expansion of parenchyma cells in the receptacle.

Hormonal Effects on Growth

Auxin. One of the earliest investigations of the effects of auxin on general fruit development was undertaken by Nitsch (1950), using strawberry fruit. He found that removal of achenes arrested receptacle growth whether they were removed at 4, 7, 14 or 21 days after pollination. When

napthaleneacetic acid (NAA) was applied to receptacles in place of achenes, parthenocarpic growth of the berries occurred, even when the achenes had been removed 4 days after pollination. Using an Avena coleoptile growth bioassay, high levels of auxin activity were found in the achenes but none was detected in the receptacle (Nitsch, 1952). When achenes were removed 4 days after pollination and auxin applied to the receptacles, strawberries reddened, whereas only those fruit already 21-days-old at the time achenes were removed developed color in the absence of auxin.

Archbold and Dennis (1984), using mass spectroscopy, found high levels of free indole-3-acetic acid (IAA) in achene tissue and smaller quantities in the receptacle tissue. Free IAA was found in achenes 4 days after anthesis and in the receptacles 11 days after anthesis. Levels in both tissues peaked at 3 ug/g dry weight by 14 days after anthesis corresponding to the maximum fruit growth rate. The IAA level in the receptacle tissue then dropped rapidly, whereas in the achenes it dropped gradually to a minimum level of 1 ug/g achene dry weight at 23 days after anthesis (red receptacle). Esterified-IAA was first detected in achenes 4 days postanthesis, but did not appear in the receptacle until 17 days post anthesis (Archbold and Dennis, 1984). High levels of amide-IAA were found in achenes at 11 and again at 23 days after anthesis (Archbold and Dennis, 1984).

Gibberellin and cytokinin. Addition of gibberellic acid (GA) to strawberry ovaries in culture induced growth only around the basal portion of the receptacle (Bajaj and Collins, 1968). No effects were noted from kinetin (CK) additions. Lis and Antowszewski (1979) removed achenes 14 to 15 days postpollination and treated half of the fruit with GA, CK, IAA or all 3 in combination. GA and CK had no influence on the accumulation of labelled $^{14}CO_2$ or ^{32}P in the receptacle tissue although IAA treatments markedly increased label accumulation, indicating that auxin evoked a sink for nutrient assimilation. Additionally, Archbold and Dennis (1985) reported that GA did not stimulate growth when applied to fruits without achenes. However, Thompson (1969) reported normal growth and ripening of parthenocarpic berries when GA was applied without auxin. Kano and Asahira (1981) reported that the addition of NAA or CK to tissue cultured receptacles delayed ripening.

Auxin, CK and GA were detected by bioassay in strawberry fruit early in development, peaking at 7 days postanthesis (Lis et al., 1978). The concentrations of all 3 were greater in the achenes than in the receptacle tissue. After 7 days postanthesis, the CK level decreased sharply in achenes and receptacles, but was present at low levels until the fruit were ripe. The GA levels were high 5 to 6 days postpollination, decreased, then increased after the fruit were ripe.

Abscisic acid. Archbold and Dennis (1984) measured abscisic acid (ABA) levels in achene and receptacle tissues. Levels of ABA, expressed as ug/fruit or ug/achene, were less than 0.1 in both tissues at anthesis and then increased with ripening to 0.45 and 0.25 for achene and receptacle tissue, respectively. The concentration of ABA was 40 to 50% of auxin levels. Lis et al. (1978) reported low ABA levels in unpollinated or 1 day postpollinated receptacles and achenes; levels increased during ripening, reaching a maximum in red fruit. Kano and Asahira (1981) found that addition of 10 $mg.liter^{-1}$ ABA stimulated the growth and ripening of tissue-cultured strawberry fruit and proposed that ABA was synthesized actively in the latter half of development after CK disappeared from the achenes.

Strawberry Fruit Compositional Characteristics During Ripening

Softening

Softening of strawberry fruit begins at the white stage of development and increases dramatically as the fruit progresses through the red stage (Culpepper et al., 1935). Electron micrographs of cortical parenchyma cells revealed separation along the the middle lamellar region (Neal, 1965). A number of studies have been done to determine the mechanism of softening. Woodward (1972) found very low levels of water-soluble pectins 14 days after petal fall. These levels increased at 21 days after petal fall (white fruit) and then reached 90% of total pectin levels at 42 days (red-ripe).

Despite the observation that water-soluble pectin levels increase as strawberry fruit ripen, no polygalacturonase (PG) activity has been detected (Neal, 1965; Barnes and Patchett, 1976; Huber, 1984). However, strawberry cell walls treated with tomato PG released pectins of similar molecular weight to those released by tomatoes, indicating the absence of a PG inhibitor in strawberries (Huber, 1984).

Barnes and Patchett (1976) reported that pectinmethylesterase (PME) activity increased during strawberry ripening but Neal (1965) proposed that strawberry softening was due to increased esterification of cell wall pectin located in layers of the middle lamella. Esterification would result in severing of cationic crosslinks and cause cell separation.

Yields of cell wall per fruit decreased with ripening (Neal, 1965), although soluble polyuronides as a percentage of the total extractable polyuronides increased (Huber, 1984). The total polyuronide content remained constant with ripening, indicating continued synthesis, while neutral sugars associated with pectin increased (Huber, 1984).

Softening of strawberry fruit is thought to be due primarily to the increased solubility of polyuronides. However, hemicellulose degradation may also contribute to softening. Knee et al. (1977) observed an increase in xylose, mannose and glucose residues in soluble polysaccharide fractions during ripening and proposed that

hemicellose polysaccharides were being degraded or released from interpolymer bonds. Huber (1984) found a shift in hemicellulose molecular weight during strawberry fruit ripening.

Cytological Changes

Electron microscopy studies revealed that receptacle tissue at petal fall consisted of both meristematic and expanding cells (Knee et al., 1977). Meristematic cells were no longer observed at 7 days after petal fall. Cells at petal fall had dense cell walls, small vacuoles, starch grains in plastids, dictyosomes, ribosomes and endoplasmic reticuli. Cells of green fruit (7 days postanthesis) had tubular proliferations of the tonoplast, which became extensive in ripe fruit. Cell walls were swollen and were traversed by protoplasmic connections between adjacent cells (Neal, 1965). At 21 days after petal fall (white), cells were expanded, vacuolated, plastids had degenerated and most of the starch had disappeared. The cortical cells, which were mostly paranchymatous, enlarged and became separated as the strawberry fruit developed and ripened (Neal, 1965). During ripening, the cells were connected only by small projections at the tips of the cells. These junctions were traversed by protoplasmic connections between the cells. Cell wall swelling was extreme, resulting in occlusion of the intercellular spaces by matrix material (Knee et al., 1977). Mitochondria appeared to be normal in ripe fruit (Knee et al., 1977).

Pigment Changes

The white stage of strawberry fruit development is regarded as the phase between the end of chorophyll/carotenoid synthesis and the beginning of anthocyanin synthesis (Woodward, 1972). Gross (1982) detected the presence of residual carotenoids in ripe fruit which originated from chloroplasts present in green fruit. Electron microscopy studies revealed that chloroplasts in ripening strawberry fruit cells disintegrated; evidently chloroplasts disintegrate during ripening without transformation to chromoplasts (Knee et al., 1977).

The major pigments synthesized in strawberry fruit are anthocyanins, primarily as pelargonidin-3-glucoside (Ryan, 1971). Anthocyanin synthesis was very low until 35 days after petal fall, then reached 75% of the final anthocyanin concentration in the following 7 days (Woodward, 1972). Pelargonidin-3-glucoside, formed from the shikimate acid pathway, is thought to be stored in the vacuole (Rhodes, 1980).

Sugars and Soluble Solids

Total sugars, consisting of reducing sugars and sucrose, are commonly measured as percent soluble solids. Soluble solids in strawberry fruit increase steadily during development (Woodward, 1972). Sugar content increased logarithmatically with development (Knee et al., 1977).

Soluble solids in ripe fruit vary from mean values of 6 to 9% depending on cultivar and cultural conditions (Duewar and Zych, 1967).

Sucrose was shown to be the major assimilate transported to the strawberry fruit (Lis and Antoszewski, 1979; Forney and Breen, 1985a). The concentration of glucose and fructose was higher than sucrose in small green and ripe fruit (Forney and Breen, 1986). Sucrose was not detected in fruit until 10 days postanthesis; concentrations increased and then decreased as fruits became red ripe. The rate of sugar exudation from intact pedicels also increased with fruit size (Forney and Breen, 1985b). The rate of sucrose transport, measured with fruit discs, proved to be highest early in development (9 days postanthesis), quickly falling to 50% of initial rates by 12-17 days postanthesis, corresponding to about half of the final growth of the fruit (Forney and Breen, 1985a).

Starch

The role of starch in strawberry fruit growth has not been determined. Long (1938) theorized that the disappearance of starch, which coincided with increased soluble solid levels, was due to hydrolysis of starch to meet the sudden high demand for sugar by fruit. Starch has been found only in the chloroplasts from green receptacle tissue, but dissappeared prior to fruit ripening (Knee et al., 1977). Degradation of starch in plastids was essentially complete by

21 days after petal fall although starch was still detected on the basis of iodine stains (Knee et al., 1977). Possibly, starch is used in early growth of the fruits until the achenes have developed sufficiently to induce rapid nutrient assimilation from the parent plant.

Proteins and Amino Acids

Other than the enzymes involved in softening, few studies have been conducted on protein changes in ripening strawberry fruit. Veluthambi and Pooviah (1984) found different polypeptide molecular weights from strawberry receptacles harvested at 0, 5, 10, 15 and 20 days after pollination. Removal of achenes resulted in new polypeptide bands. When auxin was applied, these bands did not appear. The induced polypeptides were theorized to represent inhibitors which formed when auxin was not supplied. Burroughs (1960) and Gallander (1979) reported that 79-87% of the amino acids in ripe fruit consisted of free aspartic acid, asparagine, glutamine and glutamic acid.

Titratable Acids

Citric acid is the major organic acid found in strawberries at all stages of growth (Culpepper et al., 1935). Acids, mainly citric acid, increased steadily with fruit development and decreased in overripe fruit (Woodward, 1972). The pH of fruit homogenates were 3.6, 3.3, and 3.7 for green/white, red and overripe fruits, respectively. The decrease in citric acid occurs at the same time as an increase in malic acid (Reyes et al., 1982).

Relationship of Respiration and Ethylene to Fruit Ripening

Introduction

Fruit ripening characteristics include changes in pigmentation, texture (softening), synthesis of aromatics, conversion of starch to sugars and abscission (Kader, 1985; Brady, 1987). In strawberry fruit, ripening is characterized by enlargement of receptacle tissue, softening, anthocyanin synthesis and increased levels of soluble sugars.

Fruit ripening has long been associated with changes in respiration and ethylene production. Ethylene is a plant hormone that coordinates and unifies ripening in many fruit (Abeles, 1973). Fleshy fruit are categorized into nonclimacteric and climacteric classes on the basis of respiration patterns during maturation and ripening (Yang, 1987). The climacteric fruit undergo a distinct ripening phase, signalled by a climacteric rise in respiration and increased ethylene emanation, while nonclimacteric members generally do not. Although nonclimacteric fruit may have respiratory rates comparable to climacteric fruit, the lack of a preclimacteric minimum and climacteric peak in respiration differentiates them from the climacteric group (Biale and Young, 1981). Ethylene production from nonclimacteric fruit is generally much less than from climacteric fruit. That a fruit is truly nonclimacteric is sometimes difficult to demonstrate as pathogen infection

(Oslund and Davenport, 1983) or chilling stress (Wang and Adams, 1982) can induce a respiratory rise and ethylene production not related to ripening.

The morphological source of fruit tissue is not related to respiratory behavior (Biale and Young, 1981). The drupe fruits peach and plum are climacteric whereas the cherry and olive are nonclimacteric. The fleshy tissues of the apple (climacteric) and strawberry (nonclimacteric) fruit are of receptacle derivation (Esau, 1977).

Climacteric Fruit

Climacteric fruit are so classified on the basis of a respiratory upsurge (climacteric) coinciding with the initiation of ripening which generally declines during the latter period of ripening (Watada et al., 1984). Ethylene production also increases and this increase may precede, coincide with or follow the respiratory climacteric, depending on the species and cultivar. Increased ethylene production precedes the respiratory climacteric in honeydew melon (Pratt et al., 1977), coincides with the climacteric in avocado (Eaks, 1985) and follows the climacteric in chile pepper (Gross et al., 1986). Other examples of climacteric fruits include banana, apple, pear, muskmelon, tomato (Biale and Young, 1981), and blueberry (Ismail and Kender, 1969). In the blueberry, ethylene production occurs prior to respiration in the rabbiteye fruit, while ethylene production and the respiratory climacteric are simultaneous in the highbush fruit (Shimura et al., 1986).

In climacteric fruit, softening, color development, hydrolysis of storage polysaccharides, and volatile formation are temporally associated with the respiratory climacteric (Rhodes, 1980). Harvested, immature climacteric fruit may undergo similar changes if subjected to ethylene treatment, but the quality is inferior. Ripening is thought to be initiated by ethylene in many climacteric fruits (Brady, 1987). Increased endogenous ethylene production was related to an increase in sucrose, color change and softening in loquat fruit (Hirai, 1980) and in figs (Marei and Crane, 1971). However, Jeffrey et al. (1984) reported that an increase in soluble solids and a decrease in acidity preceded the acceleration of ethylene production in tomatoes, although softening and lycopene synthesis were dependent on the gas.

The magnitude of the respiratory upsurge and ethylene production varies with the fruit species and cultivar. Avocados exhibit a very high respiration rate of 225 ml $CO_2.kg^{-1}.hr^{-1}$ during the climacteric and a correspondingly high ethylene production rate of 250 $ul.kg^{-1}.hr^{-1}$ (Eaks, 1985). Conversely, the chile pepper exhibits a much smaller increase in respiration, changing from 65 to 130 $ml.kg^{-1}.hr^{-1}$, while the ethylene peaks at only 0.6 $ul.kg^{-1}.hr^{-1}$ (Gross et al., 1986). The range of peak ethylene production in climacteric fruits varies from less than 0.1 $ul.kg^{-1}.hr^{-1}$ in the raspberry (Blanpied,

1964) to more than 400 $ul.kg^{-1}.hr^{-1}$ in the mammee apple (Akamine and Goo, 1978). The degree of the increase in ethylene production during ripening can range from a doubling of the preclimacteric level, as in the fig (Marei and Crane, 1971), to a several-hundred fold increase, as in the soursop (Paull, 1982).

Fruit maturity effects the development of the climacteric response and ripening. If harvested too immature, fruit fail to exhibit the same levels of increased respiration or ethylene production seen in more mature fruit. Pratt et al. (1977) found that 'Honey Dew' melons failed to soften or develop color if harvested less than 50% mature. Ethylene and respiratory climacterics were delayed and reached only 50% of the peaks from more mature fruit. In the case of the avocado, mature fruit will develop climacteric ethylene and respiration only after harvest. This anomaly is thought to be due to the presence of a ripening inhibitor exported from the tree to attached fruit (Tingwa and Young, 1975).

The application of ethylene or propylene, an ethylene analogue, to climacteric fruit will advance the initiation of ripening without altering the shape or magnitude of the peak (McMurchie et al., 1972). The magnitude of this effect depends on fruit maturity and the concentration of ethylene applied. Once ripening has been initiated, respiration does not return to preclimacteric levels upon removal of exogenous

ethylene. McMurchie et al. (1972) found that application of 500 ul.liter^{-1} propylene to green bananas initiated the climacteric within 10 hours after the treatment began and 24 hours before the climacteric in control fruit.

Increasing the concentration of ethylene can advance the onset of ripening. When 1.0 ul.liter^{-1} ethylene was applied to guava (Psidium guajava) fruit at the mature, preclimacteric stage, the time to the onset of ripening was decreased by 4 days (Yen and Tzong-shyan, 1986). When 10 ul.liter^{-1} was applied, the onset of ripening was further advanced by 2 days. In mature fruit, endogenous ethylene production is stimulated. Additional applications of ethylene fail to elicit any further response.

Often, the maturity stage of the fruit determines its sensitivity to exogenous ethylene. Application of 1000 ul.liter^{-1} exogenous ethylene to immature tomato fruits stimulated respiration, but endogenous ethylene production was not stimulated until after the start of ripening (McGlasson et al., 1975). Although the ripening of individual fruits was advanced, uniform ripening did not result. Conversely, application of 1000 ul.liter^{-1} ethylene to melons only 30% mature stimulated uniform ripening (Lyons and Pratt, 1964).

Extended application of high levels of ethylene can result in irreversible suppression of ripening-associated, endogenous ethylene production. Brecht and Kader (1984a)

found that ethylene production was reduced when nectarines were treated with 100 ul.liter^{-1} ethylene for 4 days, compared to a 2-day treatment. Zauberman and Fuchs (1973) showed a 50% loss in ethylene production when avocados were stored in 100 ul.liter^{-1} continuous ethylene, compared to 0 or 24 hours of ethylene treatment.

Nonclimacteric Fruit

Nonclimacteric organs lack an upsurge in respiration during ripening. Furthermore, ripening occurs without an increase in endogenous ethylene levels. Although the respiration rate of nonclimacteric fruits is often comparable to that of climacteric fruit, ethylene production from nonclimacteric fruit generally remains below 1.0 ul.kg^{-1}.hr^{-1} (Kader et al., 1985). The pattern of respiration and ethylene emanation in nonclimacteric fruits is generally one of higher rates postanthesis, gradually declining as the fruit mature (Biale and Young, 1981). Generally, nonclimacteric fruits display a gradual development of color, softening and increased soluble solids, not associated with starch breakdown, increased respiration or ethylene. Some examples of nonclimacteric fruit include watermelon (Elkashif, 1985), orange, grape, and cucumber (Biale and Young, 1981). Carambola fruit fail to show an increase in either respiration or ethylene emanation while ripening (Oslund and Davenport, 1983). The cherry exhibits a downward drift in respiration as the fruit ripen, without

increased ethylene evolution (Blanpied, 1972). Akamine and Goo (1979) failed to detect any ethylene production from the mountain apple (Eugenia malaccensis). On the other hand, cucumber fruit fail to exhibit a respiratory climacteric during maturation but do exhibit an increase in ethylene production in fruit 20 to 30 days after anthesis (Kanellis et al., 1986).

The unique response of nonclimacteric fruit to exogenous ethylene has been a useful aid in distinguishing nonclimacteric from climacteric fruit. Application of ethylene or propylene to nonclimacteric fruit results in increased respiration rates while ethylene emanation remains at low, basal levels (McMurchie et al., 1972). The magnitude of the respiratory increase depends on the fruit type and on the concentration of exogenous ethylene applied. Upon removal of ethylene, respiration returns to previous levels and reapplication of ethylene will stimulate another respiratory increase. This technique was first used with citrus. Vines et al. (1965) applied 50 $ul.liter^{-1}$ ethylene for 24 hours to Valencia oranges and observed a 70% increase in respiration. After removal of the ethylene, respiration returned to near-normal in 5 days. Treatment of lemons with 1.0 $ul.liter^{-1}$ of ethylene increased the respiration rate by 20%, while application of 100 $ul.liter^{-1}$ ethylene increased the respiration rate by more than 100% (McMurchie et al., 1972). One example where

ethylene application has provided evidence of nonclimacteric behavior is the watermelon. Mizuno and Pratt (1973) classified the watermelon as a climacteric fruit on the basis of ethylene production and respiratory rates from fruit harvested at different stages of development. However, upon application of 50 $ul.liter^{-1}$ ethylene, Elkashif (1985) found a 40% increase in respiration of mature and immature watermelons. Following removal of ethylene, the respiration rate decreased to preapplication values after 3 days. Endogenous ethylene production was not increased and ripening was not advanced. These results lead to the conclusion that the watermelon is nonclimacteric.

Generally, the onset of ripening in nonclimacteric fruit is not hastened by ethylene application. Treatment of detached cherries with 1000 $ul.liter^{-1}$ propylene for 24 hours failed to enhance color formation or endogenous ethylene production (Reid et al., 1985). Application of 500 $ul.liter^{-1}$ ethephon to grapes just before the onset of ripening advanced color formation and softening by 4 to 6 days, but delayed ripening if applied earlier in growth (Hale et al., 1970). Fudge (1930) found enhanced color formation in cranberries treated with 1000 $ul.liter^{-1}$ but no increase in respiration rates or total sugars.

Senescence may be advanced by ethylene application. Elkashif (1985) found that watermelons harvested immature or mature had accelerated cell wall breakdown, decreased flesh

firmness, and electrolyte leakage after 3 days treatment with 50 $ul.liter^{-1}$ ethylene. Pathogen infection occurred in fruit treated 8 days with ethylene. Treatment of carambolas with an ethephon dip (1000 $ul.liter^{-1}$) failed to stimulate ethylene production for more than 2 days but pathogen infection occurred after 8 days of storage (Lam and Wan, 1987).

Respiration and Ethylene in Strawberry Fruit

Generally, nonclimacteric fruit, exemplified by citrus and grape, do not exhibit rapid changes in color, softening or increased soluble sugars (Biale and Young, 1981). One exception to this generalization is the strawberry fruit, which softens and ripens within a few days, without exhibiting an increase in either respiration or ethylene.

The respiration rate of detached ripe 'Geneva' fruit held at 21°C was 56 ml $CO_2.kg^{-1}.hr^{-1}$ (Dayawon and Shutak, 1967) while detached 'Raritan' strawberries harvested at the white stage and held at 22°C respired at 70 $ml.kg^{-1}.hr^{-1}$ (Janes et al., 1978). The respiration rate of ripe 'Shasta' fruit decreased with temperature, from 120 to 10 $ml.kg^{-1}.hr^{-1}$ at 30° and 0°C, respectively (Mitchell et al., 1964). Ethylene production from ripe strawberry fruit at 20°C is generally reported to be less than 0.1 $ul.kg^{-1}.hr^{-1}$ (Kader et al., 1985). Ethylene emanation in 'Prizewinner' fruit fell from 2 $ul.kg^{-1}.hr^{-1}$ at anthesis to 0.08 $ul.kg^{-1}.hr^{-1}$ at ripeness at 20°C

(Knee et al., 1977). Application of 50 ul.liter^{-1} ethylene to white fruit failed to increase respiration (Janes et al., 1978) while 200 ul.liter^{-1} ethylene applied to detached green and white fruit failed to initiate color development (Mason and Jarvis, 1970). Although changes in respiration and/or ethylene production are associated with ripening in some fruits, the relationship between respiration, ethylene production and strawberry fruit ripening remains unknown.

A confounding factor in the above studies has been the failure of strawberry fruit to develop normally if harvested prior to the development of color (Mason and Jarvis, 1970). This complication makes it difficult to assess the validity of measurements of respiration and ethylene production from detached fruits and increases the complexity of determining the true involvement of ethylene in strawberry fruit ripening.

Ethylene Synthesis

Intermediates in Ethylene Synthesis

The sequence and identification of intermediates involved in ethylene biogenesis evolved from a series of experiments with model systems and plant tissues. Initially, Lieberman et al. (1965) used a model system of linolenic acid, with copper and ascorbic acid as catalysts to study ethylene formation. They found that methionine, added as an inhibitor of free radical reactions, could substitute for linolenic acid as a substrate and resulted in greatly

increased ethylene production. Subsequently, Lieberman et al. (1966) demonstrated that addition of methionine to apple tissue resulted in ethylene formation. The intermediates involved in the conversion of methionine to ethylene were subsequently identified as S-adenosylmethionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC) by Adams and Yang (1979) and Lursen et al. (1979). Lursen et al. (1979) found that application of ACC to soybean leaf discs resulted in ethylene formation. They predicted that ethylene biosynthesis involved the conversion of SAM to ACC. Adams and Yang (1979), following the conversion of radiolabelled methionine in apple tissue, found that SAM was converted to ACC, then ACC was converted to ethylene. All climacteric and nonclimacteric fruit thus far studied have demonstrated the ability to convert methionine to ethylene (Yang et al., 1986).

The content of ACC changes in relation to ethylene synthesis. Yang et al. (1986) found that mature but unripe apples contained 0.1 nmole.g fresh weight^{-1} ACC, increasing 300-fold during the ethylene climacteric. At the preclimacteric stage in avocado, banana and tomato fruits, ACC levels were about 0.1 nmole.g fresh weight^{-1} in each of these fruit tissues (Hoffman and Yang, 1980). During the climacteric, ACC levels increased to 45, 5 and 7 nmole.g fresh weight^{-1} for avocado, banana and tomato, respectively.

When aminovinylglycine [2-amino-4-aminoethoxy-trans-3-butenoic acid] (AVG), a specific inhibitor of ACC synthase, or amino-oxyacetic acid (AOA), an inhibitor of pyridoxal phosphate enzymes, was applied to pears or apples prior to the onset of rapid ACC synthesis, conversion of SAM to ACC was inhibited and ripening was delayed (Yang, 1985).

Enzymes Involved in Ethylene Synthesis

The enzymes regulating the conversion of methionine to ethylene were identified as methionine adenosyl transferase, ACC synthase and ethylene forming enzyme (EFE), respectively (Yang and Hoffman, 1984). The rate-limiting enzyme in ethylene synthesis was shown to be ACC synthase. Conversion of SAM to ACC in mung beans was stimulated by auxin treatment but the level of SAM did not decrease; thus conversion of methionine to SAM was not limited (Yu and Yang, 1979). Furthermore, application of ACC to tissue from several fruits greatly stimulated ethylene production, indicating that EFE was constitutive and that the level of ACC, and therefore ACC synthase, was the rate-limiting step (Cameron et al., 1979). Yang and Adams (1979) found that AVG inhibited the conversion of methionine to ACC but not of methionine to SAM or of ACC to ethylene. They concluded that ACC synthase was a pyridoxal enzyme. Boller et al. (1979) isolated ACC synthase as a soluble enzyme from cell-free extracts of tomato fruit pericarp. It was inhibited by AVG and had a molecular weight of about 55000. To date, ACC synthase has

been isolated from tomato pericarp, Cucurbita maxima mesocarp (Nakajima and Imaseki, 1986), mung bean hypocotyls (Yu and Yang, 1979), tobacco leaves (Imaseki and Watanabe, 1978), nectarine (Brecht and Kader, 1984b), citrus peel (Riov and Yang, 1982a) and cucumbers (Terai and Mizuno, 1985). Acaster and Kende (1983) reported a molecular weight of 57000 for ACC synthase isolated from wounded tomato fruit tissue. Privalle and Graham (1987) reported a molecular weight of about 50000 for the enzyme from wounded, pink tomato pericarp. Nakajima and Imaseki (1986) estimated the molecular mass of ACC synthase from winter squash mesocarp to be 160000, made up of 2 subunits, each approximately 84000 molecular weight. They calculated the specific activity of ACC synthase to be 220 MU/mg protein at 30 C, with 50 uM SAM. ACC synthase is believed to be located in the cytoplasm (Apelbaum et al., 1981).

Ethylene forming enzyme, which has not yet been isolated or fully characterized, is most likely membrane-bound, since ethylene production is disrupted when plant cells are exposed to detergents (Lieberman, 1979) or osmotic shock (Imaseki and Watanabe, 1978). Guy and Kende (1984) suggested that EFE was located on the tonoplast, and that ACC was sequestered in the vacuole, since vacuoles isolated from pea protoplasts produced 80% of the total ethylene. Application of cycloheximide (CHI), a protein synthesis inhibitor, to avocado fruit did not inhibit EFE, indicating that EFE activity is constitutive (Blumenfield et al., 1986).

Regulation of Ethylene Biosynthesis

System I and system II ethylene. The upsurge in ethylene biogenesis during climacteric fruit ripening is referred to as autocatalytic ethylene (Biale and Young, 1981). Autocatalytic ethylene is defined as that produced during the increased synthesis of ethylene triggered by exposure to ethylene (Yang and Hoffman, 1984). McMurchie et al. (1972) proposed that all tissues exhibit a low basal ethylene system which they referred to as System I. Climacteric organs have an additional system, which McMurchie et al. (1972) called System II, thought to be triggered by increased sensitivity of the tissue to its own System I ethylene. Yang et al. (1986) proposed that the endogenous ethylene produced by System I activates ACC synthase, creating more ACC, which then induced EFE and subsequent formation of more System II ethylene.

Ethylene levels remain low in nonclimacteric fruits throughout development and ripening, in contrast to the many-fold upsurge occurring in climacteric fruits. Ripening nonclimacteric tissues do not develop System II ethylene production. This difference may be due to the lack of development of tissue sensitivity to ethylene or inability to sustain System II ethylene production (McMurchie et al., 1972).

Huber and Sherman (1987) proposed that System I ethylene may initiate ripening, due to ethylene receptors. These receptors may be unmasked as inhibitors disappear, or pre-formed ripening-specific receptors may be modified by ethylene. The result, over time, is age-accruing sensitization of the tissue to ethylene.

Climacteric fruit. The regulation of ethylene production in climacteric fruit is a function of fruit maturity and species. This regulation has been found to relate to changes in fruit ACC content. Application of ethylene to intact, immature tomatoes or cantaloupes greatly increased the capacity of the tissue to convert ACC to ethylene, but did not increase the ACC content, indicating regulation by both ACC synthase and EFE (Liu et al., 1985). Application of ACC to preclimacteric apples resulted in only a 5-fold stimulation of ethylene production, compared to a 1000-fold ethylene increase during the climacteric. Apparently, some EFE must be synthesized after autocatalytic ethylene production is stimulated. Brecht and Kader (1984b) compared ACC synthase and EFE activity to ethylene production and ACC content of nectarine fruit. Detectable EFE activity preceded the activation of ACC synthase and the rise in ethylene synthesis. Yang et al. (1986) followed changes in EFE, ACC and ethylene in apples and found that EFE activity preceded changes in ACC content or ethylene production. Blumenfield et al. (1986) studied changes in ACC synthase and EFE activity, ACC content and ethylene production in

avocados. They propose that attached fruit have low levels of ACC synthase activity and ACC, which increased upon harvest. The ACC is then converted to ethylene by EFE; then the ethylene induces climacteric ethylene production. Ripening is then promoted by the higher level of ethylene.

Autoinhibition of ethylene synthesis in climacteric fruit tissue seems to be due to a blockage and decrease of EFE activity rather than of ACC synthase. In the presence of 100 $ul.liter^{-1}$ ethylene, EFE activity in nectarines dropped, although ACC synthase and ACC contents remained at similar levels relative to endogenous ethylene production (Brecht and Kader, 1984b).

Nonclimacteric fruit. Few studies have been done on the regulation of ethylene in ripening nonclimacteric fruit. Most studies have dealt with the activation of ethylene production by chilling stress or wounding. Cameron et al. (1979) applied ACC to a number of nonclimacteric fruits, including squash and bell pepper, and obtained stimulated ethylene production in these fruit. The amount of ethylene produced was dependent on both tissue types and the concentration of ACC applied. All tissues responded to 1 mM ACC; thus EFE activity was constitutive in these fruits. The occurrence of a lag phase between ACC application and ethylene production in some tissues indicated that de novo EFE synthesis may be required. To date, the ACC content has been measured in only 2 nonclimacteric fruit, citrus and

cucumber. In flavedo tissue from intact oranges, the ACC content remained constant at 0.11 nmole.g fresh weight^{-1} (Hyodo and Nishino, 1981), and decreased in whole cucumber fruit during ripening from 1.3 to 0.5 nmole.g fresh weight^{-1} (Terai and Mizuno, 1985). Ethylene biosynthesis in flavedo tissue from mandarin orange could be stimulated by aging the excised tissue for 30 hours, which was temporally related to a large increase in ACC content (Hyodo and Nishino, 1981). Ethylene synthesis could be stimulated in nonaged tissue when 1 mM ACC was applied. Cycloheximide (CHI) applied to tissue pretreated with ACC strongly inhibited the conversion of ACC to ethylene, whereas treatment with actinomycin, a transcription inhibitor, did not suppress ethylene synthesis. Apparently, synthesis of EFE was required, although mRNA synthesis had already taken place. Aged tissue pretreated with or without ACC was then treated with AVG, an inhibitor of ACC synthase. Ethylene from tissue without ACC pretreatment was suppressed, but AVG did not result in decreased ethylene from tissue with ACC pretreatment. Evidently, de novo synthesis of ACC synthase was not stimulated by the increased ethylene production brought about by ACC application, but de novo synthesis of this enzyme was required for ethylene production by aged tissue.

The activity of ACC synthase, ACC content and ethylene production increased in cucumber fruit chilled at 2.5^{o}C

then transferred to 25°C (Wang and Adams, 1982). Treatment of chilled fruit tissue with cycloheximide resulted in lowered ACC synthase activity, ACC level and ethylene production, but not with actinomycin or a-amanitin, indicating de novo synthesis of ACC synthase in response to chilling. This response was similar to that obtained with aged mandarin orange tissue.

Inhibitors of Ethylene Action and Synthesis

Several compounds have been found to inhibit or block ethylene synthesis and action. Pyridoxal enzyme inhibitors, including the vinylglycine analogs rhizobitoxine and AVG, irreversibly bind to an active site on ACC synthase, effectively blocking the conversion of SAM to ACC (Yang and Hoffman, 1984). Hydroxylamine analogs, such as aminooxyacetic acid (AOA), react with the pyridoxal phosphate coenzyme necessary for ACC synthase activity, blocking formation of more ACC. Neither analog blocks the conversion of ACC to ethylene, although cobalt and anaerobisis have been shown to inhibit the conversion of ACC to ethylene (Adams and Yang, 1979; Yang, 1985). Inhibitors of ethylene action include 2, 5 norbornadiene and silver. Norbornadiene is a volatile, cyclic olefin that competes with ethylene for a receptor binding site (Veen, 1985). Beyer (1976) discovered that silver was a potent inhibitor of ethylene action. Silver has been found to inhibit System II ethylene synthesis and lycopene formation in tomato fruit (Hobson et al., 1984;

Saltveit et al., 1978), and prevents senescence in cut flowers, but the exact mechanism of action is unknown (Veen, 1986).

Summary

Strawberry fruit growth is composed of achene development and receptacle enlargement. Assimilate transport from the plant to the developing fruit takes place through the vascular bundles. Biochemical changes such as softening and anthocyanin synthesis begin just prior to the white stage. Respiration and ethylene studies of strawberry fruit have been done on ripe or nearly-ripe fruit. Ethylene levels are very low, while respiration rates are high relative to other fruit.

Fleshy fruit have been classified as climacteric or nonclimacteric on the basis of respiration patterns during ripening. Climacteric fruit exhibit a decreased respiration rate, followed by a respiratory upsurge, just before and during ripening. Ethylene production exhibits a similar pattern. When exogenous ethylene is applied to these fruit at the preclimacteric stage, the onset of ripening is hastened. Nonclimacteric fruit show no variation in ethylene production or respiration during growth, other than a general downward drift from anthesis through ripening. Ripening changes are associated with ethylene in climacteric fruit, but researchers have thus far failed to establish the role of ethylene in the ripening of nonclimacteric fruit.

It is proposed that the difference between climacteric and nonclimacteric fruit ripening is related to differences in ethylene production and sensitization. Climacteric fruit are capable of autocatalytic ethylene production, which may be the result of increased tissue sensitivity to basal ethylene levels present throughout growth. Nonclimacteric fruit either do not become sensitized to basal ethylene levels or are unable to sustanin higher levels of ethylene production. Nonclimacteric fruit are capable of forming EFE and ethylene from exogenously applied ACC.

Strawberry fruit exhibit marked changes in growth, pigmentation, texture and flavor during ripening but the role of ethylene, if any, in strawberry fruit ripening remains unknown.

CHAPTER III
DEVELOPMENT AND EVALUATION OF AN IN VITRO SYSTEM TO STUDY DETACHED STRAWBERRY FRUIT RIPENING

Details on the induction and regulation of ripening have been largely derived from studies with climacteric fruit (Biale and Young, 1981). If detached at a mature stage, these fruit generally will continue to ripen normally, exhibiting increased respiration and ethylene production which can be monitored postharvest. Direct study of nonclimacteric fruit has been limited because normal ripening generally does not continue following detachment (McGlasson, 1985). Furthermore, respiration and especially ethylene production do not change dramatically during ripening, making it difficult to identify the initiation of ripening events.

Strawberries have been classified as nonclimacteric fruit because of the lack of a respiratory increase during ripening. Additionally, no rise in ethylene occurs during ripening (Biale and Young, 1981). Although anthocyanins can appear in strawberries detached preripe, textural changes are abnormal and soluble solids do not increase (Austin et al., 1960).

The ability to harvest strawberry fruit at an early stage of development and promote normal ripening under

controlled, *in vitro* conditions would allow experimentation directed towards elucidating details of the physiology of ripening of nonclimacteric fruit. Loewus and Kelly (1961) placed green strawberries having a small portion of the attached peduncles into vials containing water and radiolabelled D-galacturonic acid to study uptake and utilization of this hexuronic acid. Fruit developed to the pink color stage; the galacturonic acid was incorporated and used in the receptacle tissue for the synthesis of ascorbic acid and pectin. Further studies employing this approach hav not been performed with strawberries. In a study of the response of cherry fruit to propylene and silver, Reid et al. (1985) pretreated cherry fruit by immersion of attached peduncles into a silver thiosulphate solution. Such *in vitro* systems have long been employed for the study of cut flower physiology. Vase solutions in the simplest form contain a germicide and sucrose and these treatments have resulted in increased dry weights and the promotion of flower longevity for carnations (Reid et al., 1980b), roses (Ferreira and DeSwardt, 1980) and other flowers (Halevy and Mayak, 1979). The response of cut flowers to vase solutions depends on species, cultivar and flower maturity at harvest. Based on the success of vase solutions with cut flowers and the system used by Loewus and Kelly, it seemed that the morphology of strawberries might lend themselves to treatment analogous to cut flowers.

The objectives of this study were to investigate the conditions necessary to promote growth and ripening of detached strawberry fruit, and to compare the parameters of fruit maintained *in vitro* to those fruit maintained in the field.

Materials and Methods

Short-day strawberry (*Fragaria* X *anassasa*) cultivars 'Douglas' and 'Pajaro' were grown in field plantings in Dover and Gainesville. Blossoms were tagged at anthesis and fruit harvested with intact peduncles from 6 to 22 days postanthesis. Immediately after the fruit with peduncles were detached, they were placed in plastic bags, sealed, and placed on ice during transport from the field to the laboratory. Fruit were stored up to 3 weeks at $1^{o}C$ without detrimental effects.

In Vitro Growth Experiments

The cultivars 'Douglas' and 'Pajaro' were harvested at 14 days postanthesis, at a time when fruit maturity, expressed as a percentage based on the total number of days from anthesis to full ripeness, was approximately 52%. Vase solution composition was based on that commonly employed for cut flowers (Halevy and Mayak, 1981) and consisted of autoclaved distilled water with 200 $ug.liter^{-1}$ hydroxyquinoline hemi-sulfate (HQS) with or without 3% (88 mM) sucrose (Fisher analytical grade). The pH of each solution was 4.0. Effects other than antimicrobial have been

reported for HQS (Halevy and Mayek, 1979). However, omission of this agent resulted in prolific microbial growth and loss of fruit weight. Twelve fruit were used for each treatment. Initial fruit fresh weights were matched between treatments. The average fruit fresh weights were 5.44±1.01g and 5.63±1.10g for 'Pajaro' and 'Douglas', respectively.

After trimming peduncles to a uniform length of 55 mm with a scalpel, each fruit was placed in an autoclaved, 18.5 ml scintillation vial (Kimble 27 1/4 x 55) filled with 16 ml of solution and covered with parafilm which was slit to accommodate the peduncle. The base of each peduncle was recut to remove 1 to 2 mm every 2 to 3 days during all experiments. The daily loss in fresh weight due to peduncle trimming was about 0.1% fresh weight. Fresh weight was recorded every 1 to 3 days throughout the experiments. Duplicated experiments were conducted on lab benches under fluorescent lights (12 hours) at 23 ± 3°C. Fruit surface color was measured using a Hunterlab Colorquest color difference meter. Values were recorded in the L (black to white); a (green to red); b (blue to yellow) system using a 25 mm aperture calibrated with white (No. 1077, x=81.7 y=86.53 z=93.98) and gray (No. 1077, x=51.37 y=54.64 z=59.20) standards. Values for L, a, b corresponding to strawberry color stages are listed in Table 3-1.

Influence of Fruit Age at Harvest on In Vitro Development

'Pajaro' fruit were harvested at 14 and 20 days post-anthesis (approximately 54% and 77% maturities, respectively). Twelve fruit of each age were placed via peduncles in solutions containing 200 ug·liter^{-1} HQS with or without 88 mM sucrose. Average initial fresh fruit weights were 2.95±.41 g and 13.65±2.30 g for 14 and 20 day fruit, respectively. Further experiments were conducted with 'Pajaro' fruit to examine the effects of fruit age on *in vitro* response. Fruit at 6, 12 and 18 days postanthesis (25%, 53% and 78% mature, respectively) were harvested and placed in solutions consisting of 88 mM sucrose and 200 ug·liter^{-1} HQS. Solutions and measurements were made as described above. Receptacle length, defined as the distance from the calyx to fruit tip, and maximum diameter were determined with a Vernier caliper to the nearest 0.1 mm.

Influence of Fruit Order on In Vitro Development

Primary, 2^{o} and 3^{o} 'Pajaro' fruit of the same anthesis date were harvested from the field at 14 days postanthesis (58% mature). Twelve fruit per order were placed in the 88 mM sucrose with 200 ug·liter^{-1} HQS solutions. Measurements were conducted as described above. Average initial weights were 3.47±.75 g, 2.68±.36 g, and 1.14±.11 g for 1^{o}, 2^{o} and 3^{o} fruits, respectively.

Comparison of Field to In Vitro-Ripened Fruit

'Pajaro' fruit tagged on the same date of anthesis were harvested from the field at 12 and 19 days postanthesis and placed into *in vitro* sucrose solutions after harvest as

Table 3-1. Relationship between the color of *in vitro* grown strawberry fruit and HunterColorlab L, a, b values.

Fruit Color[z]	Value		
	L	a	b[y]
Green (G)	50±4	-7±1	17±2
White (W)	61±5	-4±2	16±1
Pink$^-$ (P$^-$)	52±4	-1±2	14±2
Pink (P)	45±4	9±3	14±2
Red$^-$ (R$^-$)	37±3	14±2	14±2
Red (R)	31±2	20±3	11±3
Red$^+$ (R$^+$)	22±4	30±5	10±2

[z]Pink$^-$=less than 1/4 pink on fruit; red$^-$= light red; red$^+$=deep red. Each value represents the mean of 24 measurements, ± SD.

[y]L=value (black to white), a=hue (green to red), b=chroma (blue to yellow).

described above. Length and diameter measurements of twelve tagged fruit left attached to the plants were taken at 2 to 3 day intervals from 12 days postanthesis until harvest at 24 days (fully ripe). Final weights and color values of fruit harvested at 24 days postanthesis were compared to those ripened *in vitro*.

Results

In Vitro Growth Experiments

Figure 3-1 illustrates the cumulative growth of strawberry fruit harvested 14 days postanthesis and placed into vase solutions. 'Pajaro' fruit consistently showed significantly more weight gain than did 'Douglas' fruit, regardless of the presence of sucrose (Fig. 3-1). 'Pajaro' fruit in solutions without sucrose gained 30% more weight than 'Douglas' fruit in solutions with sucrose. This difference increased to 300% when 'Pajaro' fruit were grown in solutions containing sucrose. 'Douglas' fruit grown without sucrose failed to gain weight.

Color development in 'Pajaro' fruit advanced more than in 'Douglas' fruit. Although 'Douglas' fruit grown in the presence of sucrose reached the white color stage at the same time as 'Pajaro' fruit grown in sucrose, further color development in 'Douglas' fruit was suppressed. 'Pajaro' fruit grown with sucrose developed to a full red color while still gaining weight, whereas 'Douglas' fruit grown with sucrose began to lose weight after reaching the pink color

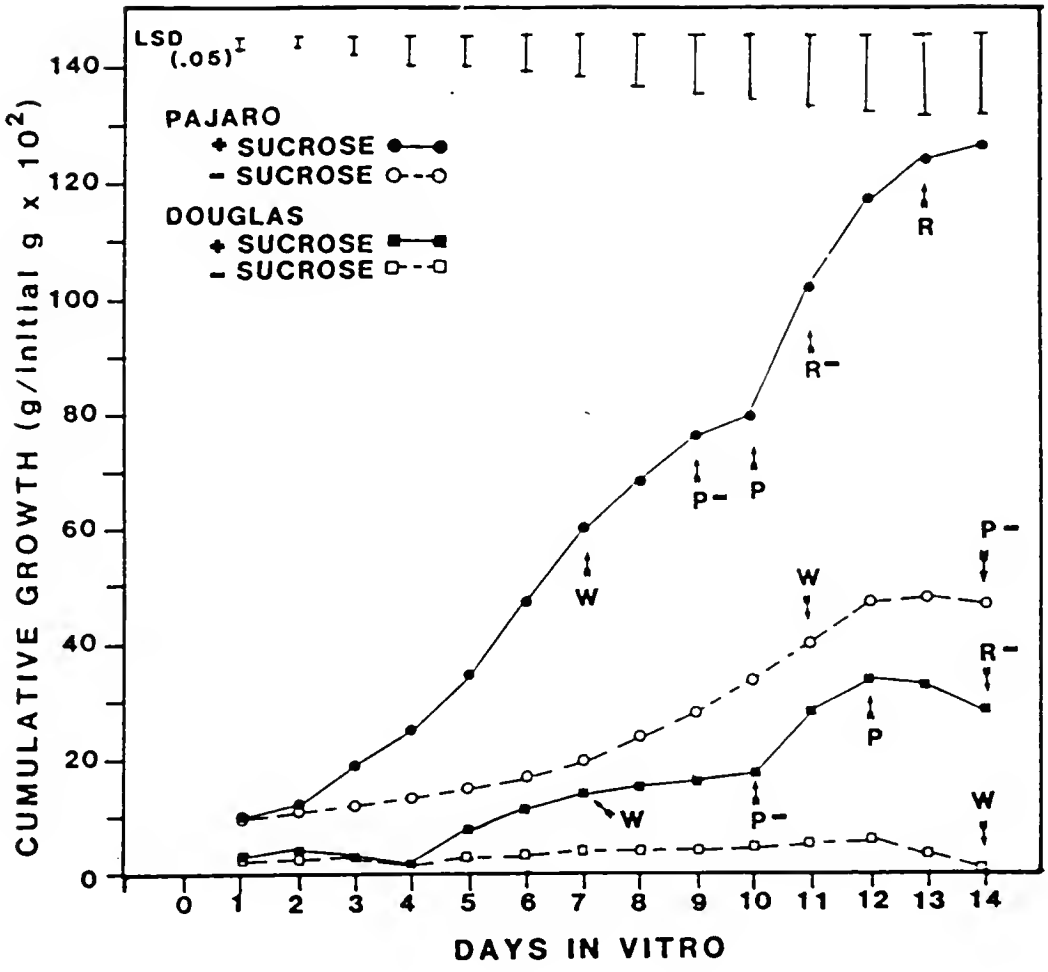

Figure 3-1. Effect of cultivar and sucrose on cumulative fruit growth *in vitro*. 'Pajaro' or 'Douglas' fruit were placed in water with 200 ug.liter^{-1} HQS, with or without 88 mM sucrose. Day of color change is indicated by W (white), P (pink) or R (red). Vertical bars represent LSD between cultivars and sucrose levels for each day.

stage. Color formation in 'Pajaro' fruit grown without sucrose was delayed by 4 days relative to 'Pajaro' fruit grown with sucrose and was nonexistent for 'Douglas' fruit grown without sucrose. Because of the poor *in vitro* performance of the 'Douglas' fruit, only 'Pajaro' fruit were used for subsequent experiments. Despite its poor growth *in vitro*, 'Douglas' fruit in the field were larger than 'Pajaro' fruit and developed color normally.

Influence of Age on Fruit Growth and Ripening In Vitro

'Pajaro' fruit harvested at 20 days (white; 77% mature) or 14 days (green; 54% mature) postanthesis responded differently to the presence of sucrose (Fig. 3-2). White fruit provided with sucrose showed less weight gain but had accelerated color development compared to white fruit held in solutions not containing sucrose (Fig. 3-2A). Color formation in white fruit grown without sucrose was delayed by 3 days compared to fruit grown with sucrose. Once color formation was initiated, the rates of color development were the same for white fruit grown with or without sucrose. The cumulative weight gain of white fruit grown either in the presence or absence of sucrose did not exceed 0.40 g/initial g fruit fresh weight.

Fruit harvested at the green stage gained 400% more weight when grown with sucrose (Fig. 3-2B). Fruit grown with sucrose exhibited the greatest weight gain concomitant with ripening and continued to gain weight as color

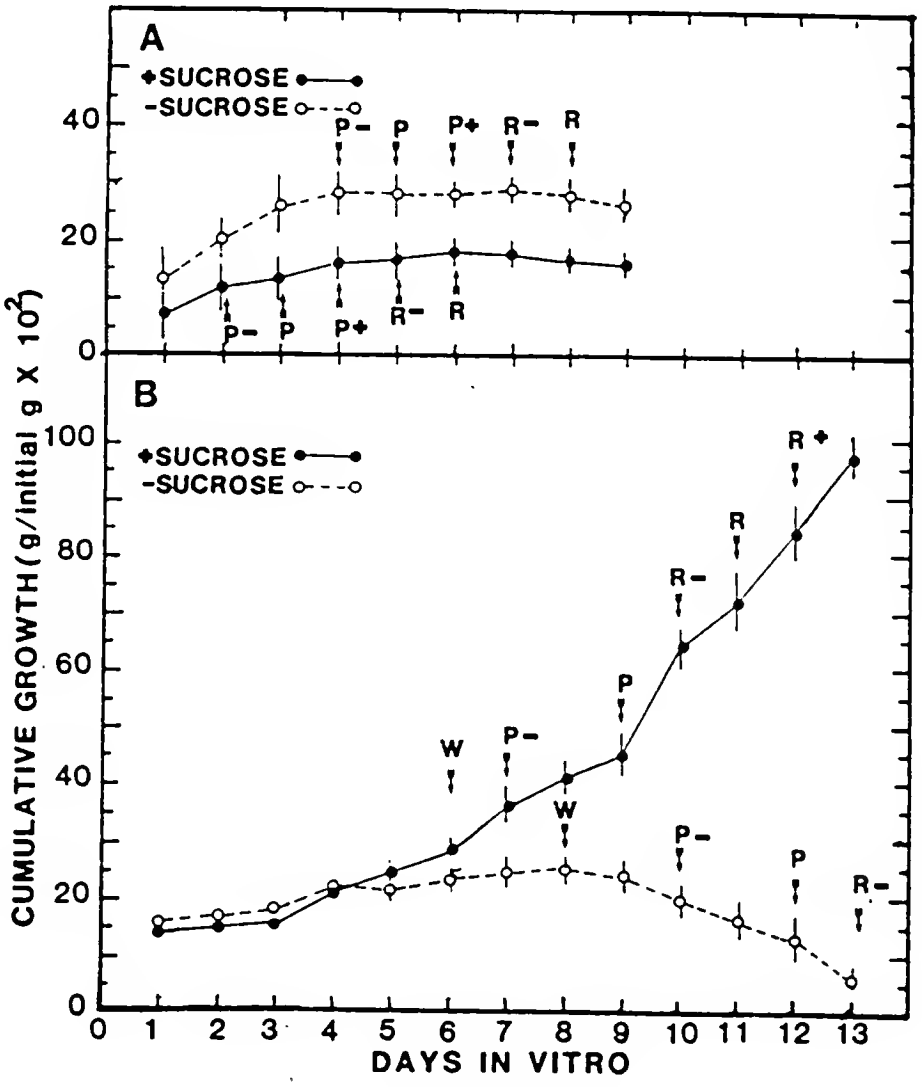

Figure 3-2. Effect of age and sucrose on cumulative growth of 'Pajaro' fruit *in vitro*. Fruit were harvested 20 days post-anthesis (white) (A) or 14 days post-anthesis (green) (B) and placed in vials with or without 88 mM sucrose. Day of color change is indicated by W (white) or R (red). Vertical bars represent standard error of the mean (SE).

intensified. Fruit grown without sucrose exhibited a decrease in weight as fruit ripened.

Fruit placed *in vitro* as early as 6 days postanthesis (25% mature) had total weight gains similar to fruit harvested 12 days postanthesis (53% mature) (Fig. 3-3). Fruit placed *in vitro* at 18 days postanthesis (78% mature) gained relatively less cumulative weight than fruit placed in vase solutions at either 6 or 12 days postanthesis. During the first day *in vitro*, the weight gain was 0.10 g/initial g for fruit at all ages. After 6 days *in vitro*, fruit harvested at 18 days postanthesis were red and fruit harvested 12 day postanthesis had gained 60% more weight relative to fruit harvested 6 days postanthesis. Fruit harvested 18 days postanthesis gained less than 0.4 g/initial g *in vitro*. Fruit placed *in vitro* at 12 days postanthesis exhibited a large increase in growth rate after 8 days *in vitro*, concomitant with the start of color development. The weight gain of these fruit at this time was 0.7 g/initial g. During ripening in the following 6 days, these fruit gained an additional 0.5 g/initial g. Fruit placed in solutions at 6 days post-anthesis ripened after 18 days *in vitro*. After 13 days *in vitro*, these fruit had gained 0.6 g/initial g, then gained another 0.6 g/initial g as they developed color in the following 6 days. Unlike the fruit placed in vase solutions at 12 days postanthesis, fruit placed *in vitro* at 6 days postanthesis did not exhibit a large increase in growth rate concomitant with ripening.

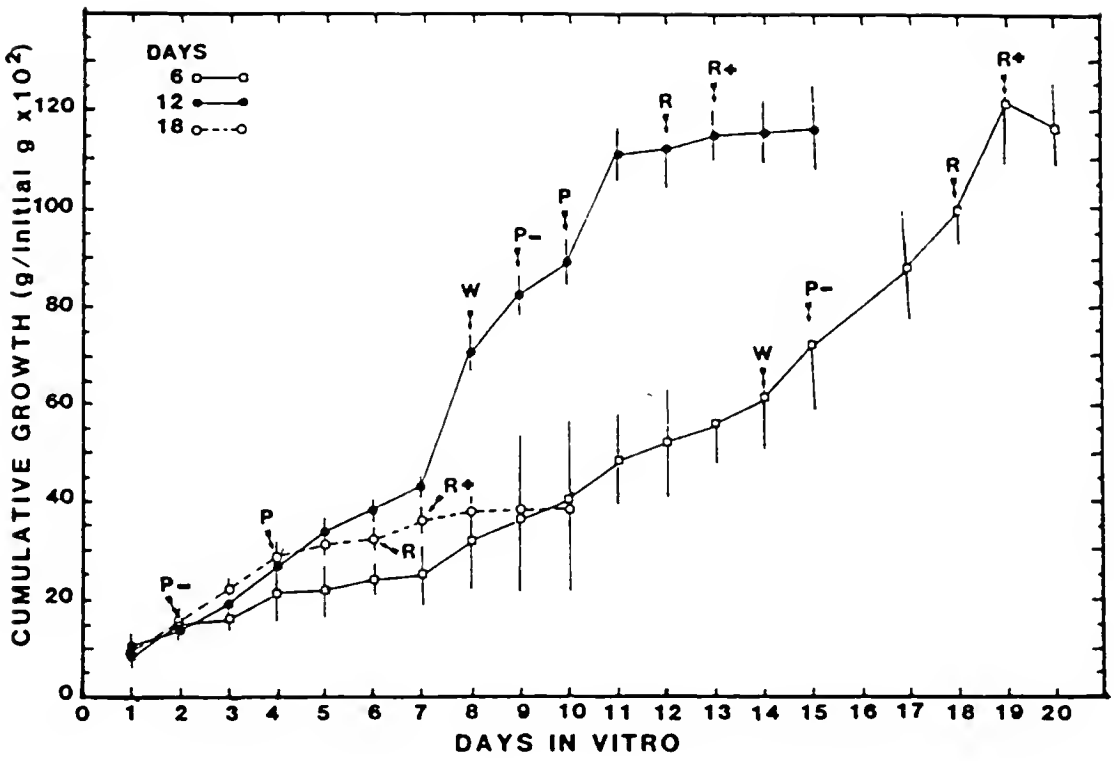

Figure 3-3. Effect of fruit age on cumulative growth in vitro. Fruit were harvested at 6 days, 12 days or 18 days post-anthesis and placed in vials containing 88 mM sucrose. Day of color change is indicated by W (white), or R (red). Vertical bars represent SE of 12 fruit.

Regardless of the initial fruit age and days *in vitro*, all fruit ripened within 24 days from anthesis (Fig. 3-3). Fruit initiated ripening (white stage) at 20 days post anthesis and the number of days between white and red stages was the same for fruit harvested at 6, 12 or 18 days post anthesis. The rate of growth, calculated as g/initial g/day, was greatest for fruit placed *in vitro* at 12 days postanthesis and least for fruit grown *in vitro* 18 days postanthesis (Table 3-2). However, final fruit size was greatly influenced by initial fruit size. Fruit harvested after 18 days postanthesis had greater initial and final fresh weights, lengths and diameters than fruit harvested after 12 or 6 days postanthesis. Fruit harvested 18 days postanthesis had the greatest rate of weight gain of 0.329 g/day.

Comparison of In Vitro Response between Fruit Orders

Primary and 2^{o} fruit generally did not differ in daily or total weight gain (Fig. 3-4). Fruit from each inflorescence position gained about 0.15 g/initial g during the first day in the vase solution, and obtained a final cumulative weight increase of 0.9 to 1.0 g/initial g. Tertiary fruit ripened at the same time as 1^{o} and 2^{o} fruit, but gained porportionately more weight as they changed color from white to red. The growth rates *in vitro* were similar between 1^{o}, $2^{o}$and 3^{o} fruit until the white

Table 3-2. Effect of age on *in vitro* growth of 'Pajaro' strawberries harvested and placed in solutions at 6, 12 or 18 days after anthesis.[z]

Days PA	Fruit Size and Weight						Rate of Growth		
	Initial Fruit Size			Final Fruit Size			g/initial g x 10^2	g/day	g/day
	Weight	Length	Diameter	Weight	Length	Diameter			
	(g)	(mm)		(g)	(mm)				
6	0.85±.06	11.9±.7	8.9±.2	1.88±.16	16.5±.6	14.0±.6	6.37±.11	.054±.031	
12	1.90±.12	18.5±.4	13.7±.2	4.10±.24	22.4±.5	18.5±.3	9.67±.18	.183±.030	
18	7.01±.61	32.1±.6	21.7±.6	9.64±.51	32.8±.5	23.8±.7	4.75±.12	.329±.072	

[z]Each value represents the mean of 12 fruit plus or minus the standard error of the mean

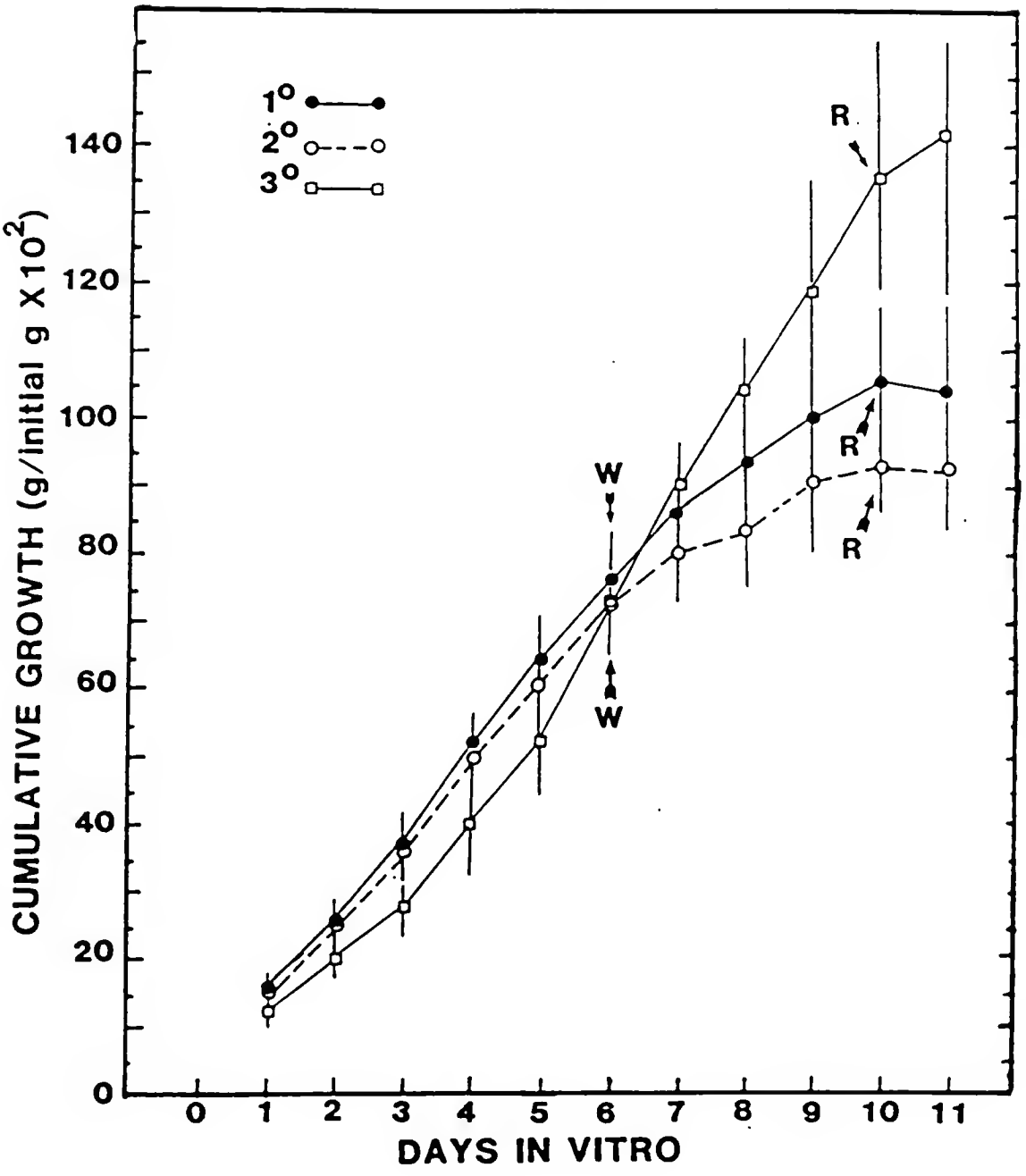

Figure 3-4. Effect of fruit order on cumulative growth *in vitro*. Fruit harvested 14 days post-anthesis. Primary, secondary, and tertiary fruit turned white (W) or red (R) on the same day. Vertical bars represent SE of 12 fruit.

Table 3-3. Rates of growth in vitro for primary, secondary or tertiary fruit order.

Time Interval	Fruit Order		
	Primary	Secondary	Tertiary
(Days)	(g/initial g/day x 10^2)		
0 to 6	12.7±.16	12.1±.14	12.0±.11
6 to 11	5.4±.09	3.9±.06	13.9±.13

Table 3-4. Comparison of[z] *in vitro* fruit weight gain and ripening to field fruit.

	Days PA	Initial Weight	Final Weight	FW /Field FW	Dry Weight	Color L	Color a	Color b
		(g)	(g)	(%)	(g)			
In Vitro	12	2.88± .32	6.94± .73	55	0.46±.08	41±3.1	17±2.0	14±1.7
	19	10.48±1.00	11.36±1.03	90	0.89±.11	38±1.2	20±1.4	13±1.1
Field	23	----	12.64±1.60	--	0.96±.13	39±2.3	19±2.7	12±1.5

[z]Each value represents the mean of 12 fruit plus or minus SE. PA=post-anthesis; FW=final weight. Days from anthesis to ripe were 23 for both in vitro and field-ripened fruit.

L,a,b represent value (black to white), hue (green to red) and chroma (blue to yellow), respectively.

Table 3-5. Comparison of length and diameter of *in vitro* and field-ripened fruit of the same anthesis date.[z]

Fruit Source	Days PA	Fruit Size: Initial Length	Initial Diameter	Final Length	Final Diameter	[Final-Initial] Length	[Final-Initial] Diameter
		(mm)		(mm)		(mm)	
In Vitro	12	24.1±.4	16.6±.2	29.8±.5	23.6±.2	5.7	7.0
Field	12	22.6±.3	15.1±.2	38.4±.7	28.2±.3	15.8	13.1
In Vitro	19	37.2±.5	26.4±.7	37.3±.6	27.1±.4	0.1	0.7
Field	19	34.7±.6	25.4±.3	38.4±.8	28.2±.3	3.7	2.8

[z]Each value represents the mean of 12 fruit, plus or minus SE.

stage of color development (Table 3-3), then the growth rates of 1^{o} and 2^{o} fruit slowed, while the 3^{o} growth rate remained constant.

Comparison of In Vitro Growth to Field Growth

Fruit harvested at 12 days postanthesis and ripened in vase solutions had lower final fresh or dry weights than fruit harvested at 19 days and ripened in vase solutions or fruit ripened in the field (Table 3-4). Fruit placed *in vitro* at 12 days postanthesis achieved 55% of the final weight of fruit ripened in the field, while fruit placed *in vitro* at 19 days postanthesis obtained 90% of field fruit weight. No differences in color values were found between fruit ripened in the field or *in vitro*, regardless of number of days *in vitro*. Fruit ripened in the field had greater length and diameter than fruit harvested at 12 or 19 days postanthesis and ripened *in vitro* (Table 3-5). Fruit placed *in vitro* at 19 days postanthesis increased very little in either length or diameter, relative to the field-ripened fruit.

Discussion

These results demonstrate that floriculture techniques employed for increased cut flower longevity provide a feasible and satisfactory method for examining the characteristics of strawberry fruit growth and ripening. Strawberry fruit could be ripened successfully *in vitro* when grown in vase solutions containing sucrose and HQS. Similar

success has been reported for floral crops harvested and held in solutions containing sucrose and an antimicrobial agent, such as HQS. Hydroxyquinoline salts lower the pH of water and prevent microbial growth in vase solutions (Marousky, 1971) and reduce vascular resistance to water flow (Burdett, 1970). Addition of sucrose to vase solutions promotes the longevity of roses (Ferreira and DeSwardt, 1980), carnations (Reid et al., 1980b) and other cut flowers (Halevy and Mayak, 1981). Sucrose was essential for best growth and ripening of strawberries harvested at the green stage, Sucrose is the major assimilate translocated to strawberry fruit (Forney and Breen, 1985b). Studies with roses employing vase solutions containing radiolabelled sucrose indicate that sugars are used for protein and carbohydrate synthesis (Paulin, 1986).

Sucrose also was essential for optimum color development in strawberry fruit grown in vitro. Enhanced anthocyanin development in the presence of sucrose has been reported for carnations held in vitro (Lee et al., 1980) and for strawberry leaf discs (Creasy et al., 1965). In strawberry leaves, most of the anthocyanin precursors are derived from the shikimate acid pathway and develop in response to ultraviolet light (Rhodes, 1980), as is pelargonidim, the predominant anthocyanin in strawberry fruit (Fuleki, 1969).

The in vitro growth performance of strawberry fruit was greatly dependent on cultivar and fruit age. The failure of 'Douglas' fruit to gain weight or ripen in vitro could have

been due to several factors. The achenes blackened after 5 days *in vitro*, whereas the achenes on 'Pajaro' fruit remained green. Viable achenes provide auxin, which stimulates enlargement of receptacle tissue (Archbold and Dennis, 1985; Nitsch, 1950). The failure of 'Douglas' fruit to exhibit significant weight gains may also have been due to problems with microbial stem blockage or the physiological composition of the peduncles. Microorganism blockage of vascular tissue has been shown to greatly reduce the vase life of cut carnation flowers, while the effect of these microorganisms is species-specific (Zagory and Reid, 1986). Van Meeteren (1978) found that stem break in cut gerbera flowers was due to reduced water uptake caused by bacterial activity, and that the incidence of stem break was dependent on the cultivar. Certain rose cultivars are very susceptible to "bent neck" which is caused by a lack of stem lignification and results in a lack of water uptake (Zieslin et al., 1978). Color development in 'Douglas' was consistently initiated at the proximal end and proceeded towards the tip (distal end). Fruit exhibited weight losses before full color was obtained. In 'Pajaro' fruit held in sucrose solutions, initiation of surface color occurred in a random fashion and weight gain continued well into color development.

Fruit placed in vase solutions at 12 to 14 days post-anthesis (50 to 60% mature) exhibited the most growth and color development when sucrose was provided. Fruit

harvested at 18 to 20 days post-anthesis (75 to 80% mature) did not respond to added sucrose in terms of either weight gain or color development but did ripen normally. Evidently, fruit harvested at this stage had assimilated sufficient carbohydrate to permit normal anthocyanin development and had already achieved most of their growth. The less mature green fruit, which failed to either gain weight or develop red color when held in solutions without sucrose, apparently lacked sufficient carbohydrate levels necessary for anthocyanin formation or sustained growth.

The facts that green strawberry fruit provided with sucrose gained more weight and that sucrose hastened the ripening of both green and white fruit is evidence that fruit were assimilating and metabolizing sucrose in a manner similar to cut flowers. Both the xylem and phloem of roses are involved in the translocation of sucrose from vase solutions (Ho and Nichols, 1975); these tissues may also have translocated sucrose to strawberry receptacles.

Although fruit harvested at 6 days postanthesis did ripen, they exhibited a lower growth rate than fruit harvested at later stages of development. The low weight gain of these fruit may have been due some disintegration of the peduncles in the vase solutions.

Competitive differences between fruit on the plant could be largely overcome by placing fruit *in vitro*. Tertiary fruit exhibited a greater growth rate between the white and

red stages of ripening than did 1^{o} or 2^{o} fruit. The 3^{o} fruit initially weighed 60% less than 1^{o} fruit, and may have required more time *in vitro* to overcome the suppression of growth induced by 1^{o} and 2^{o} fruit competition prior to harvest. Janick and Eggert (1968) found that removal of 1^{o} fruit resulted in increased 2^{o} fruit weight. Fruit of the same anthesis date placed *in vitro* ripened simultaneously regardless of fruit order.

Final fruit size *in vitro* was ultimately controlled by the initial size but color development was not. Lower initial weights resulted in lower final weights within fruit age. The lower final fruit weights from *in vitro*-ripened fruit compared to field-ripened fruit has also been noted in tissue-cultured strawberries (Bajaj and Collins, 1968). Detachment of the fruit may have resulted in vascular system injury and lower subsequent solution uptake. The vase solutions employed here also lacked other nutrients and hormones which may enhance growth of attached fruit. Vase solutions with 1% and 5% sucrose were tried (data not shown), but 1% sucrose resulted in nonuniform fruit growth while 5% sucrose resulted in calyx necrosis.

The onset of ripening was not delayed for fruit grown *in vitro*, even in fruit harvested as young as 6 days postanthesis and weighing as little as 0.85 g fresh weight. Evidently, initiation of ripening was not dependent on the attainment of a critical fruit weight and use of an *in vitro*

system did not interfere with the changes required for the expression of ripening.

Conclusions

Successful ripening of immature strawberry fruit was accomplished by placing fruit with attached peduncles in vase solutions composed of HQS and sucrose. Fruit cultivar and maturation stage are the important variables which must be considered when using the *in vitro* system. 'Pajaro' fruit harvested at 50 to 60% maturity exhibited the greatest and most uniform weight gain when placed in vase solutions containing 200 $ul.liter^{-1}$ and 88 mM sucrose. Although the final fruit weight of *in vitro* ripened fruit was less than that of field-ripened fruit, color developed *in vitro* at the same rate and to the same levels as field fruit.

CHAPTER IV
RESPIRATION AND ETHYLENE PRODUCTION IN DEVELOPING STRAWBERRY FRUIT GROWN *IN VITRO*

Fleshy fruits are categorized into nonclimacteric and climacteric classes on the basis of respiration patterns during maturation and ripening (Yang, 1987). The climacteric fruit undergo a distinct rise in respiration and ethylene emanation, whereas nonclimacteric members do not. Conclusively identifying fruit as nonclimacteric can sometimes prove difficult. Fruit subjected to pathogen infection (Oslund and Davenport, 1983), chilling temperatures (Wang and Adams, 1982) and other forms of stress can exhibit elevated respiration and ethylene production unrelated to ripening.

Application of exogenous ethylene has been shown to induce respiratory and other physiological responses in both climacteric and nonclimacteric fruit types. McMurchie et al. (1972) found that application of propylene, an ethylene analogue, to bananas would advance ripening and the onset of the respiratory and ethylene climacterics without altering the magnitude of the respiratory peak. When propylene was applied to lemons and oranges, respiration was enhanced but ripening was not advanced and endogenous ethylene production was not enhanced over the low basal levels.

Beyer (1976) discovered that silver nitrate was a potent inhibitor of ethylene action. Subsequent studies were made with silver thiosulfate (STS), which is more readily trasnsported in plant tissue (Veen, 1983). Silver has been shown to inhibit wilting of canrnation flowers (Reid et al., 1980a,b) and to induce male flowering in cucumbers (Den Nijs and Vissen, 1980). Lettuce roots exposed to ethylene in the presence of STS continued normal elongation (Abeles and Wydowski, 1987). Silver applied to intact tomato fruit inhibited lycopene and polygalacturonase synthesis (Hobson et al., 1984), but long-term treatment with STS stimulated ACC synthesis and conversion of ACC to ethylene (Atta-Aly et al., 1987), but the exact mechanism by which silver blocks ethylene action remains unknown.

Attempts to advance the ripening of strawberry fruit, reportedly a nonclimacteric fruit type, with ethylene have been unsuccessful. Janes et al. (1978) found that treating detached white strawberry fruit with 50 ul.liter^{1} for 25 hours failed to enhance color development or stimulate respiration. Similarly, postharvest application of 200 ul.liter^{-1} ethylene for 24 hours to detached green or white fruit did not initiate color development (Mason and Jarvis, 1970). The ability of exogenous ethylene to stimulate endogenous ethylene production was not studied.

A confounding factor in the above studies has been the failure of strawberry fruit to develop normally if harvested

prior to ripeness (Mason and Jarvis, 1970). This complication makes it difficult to determine the true involvement of ethylene in strawberry fruit ripening.

In the previous section (Chapter III), it was demonstrated that strawberry fruit detached immature and placed via peduncles into vase solutions of 3% sucrose were able to grow and ripen. This system provided a means of studying the long-term changes in respiration and ethylene production of strawberry fruits during growth and ripening under controlled conditions.

The objectives of these experiments were to characterize respiratory and ethylene changes of developing strawberry fruit and to study the effects of ethylene application on ripening of detached strawberry fruit maintained *in vitro*.

Materials and Methods

Respiration and Ethylene Production Measurements

'Pajaro' strawberry (*Fragaria* X *ananassa* Duch.) fruit were tagged at anthesis and harvested from field plantings in Gainesville and Dover, Florida. Fruit were harvested at green, green-white, white, pink and red stages of color development. Fruit maturation at each developmental stage was calculated as the percent of days from anthesis to field-ripeness (Fig. 4-1). Fruit were cut with peduncles attached, placed in plastic bags and transported on ice to the laboratory. Fruit not used immediately were stored at $1^{o}C$. Storage for up to 4 weeks at $1^{o}C$ did not impair in

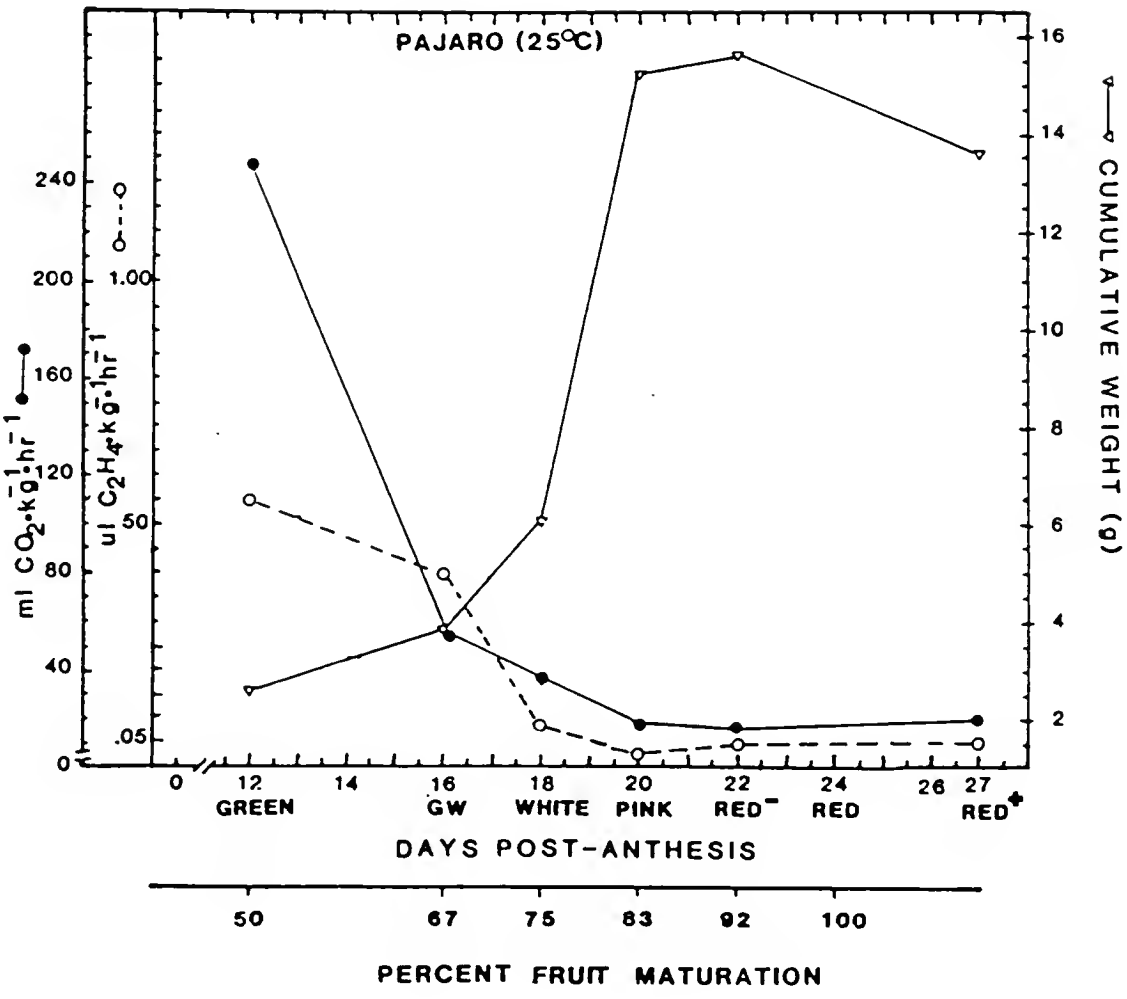

Figure 4-1. Changes in respiration and ethylene in strawberry fruit harvested at selected stages of development. Percent maturation is a function of days from anthesis (green) to the red stage of color development. SE bars are within symbols.

vitro fruit growth upon removal from storage. Six fruit per color stage were selected to measure the capacity of fruit to produce CO_2 and ethylene at different developmental stages. Fruit were placed via peduncle into individual 16 ml scintillation vials containing 15 ml sterile distilled water to alleviate water stress. Each vial with a single fruit was placed in a 252 ml jar and sealed for 1 hour at 25°C ± 3°. Respiration measurements, determined as CO_2 production, were made after 0.5 hr by sampling 0.5 ml of atmosphere with gas-tight syringes and injecting into a gas chromatograph equipped with a thermal conductivity detector (Fisher 1200). Levels of ethylene were determined by sampling 0.5 ml of atmosphere with airtight syringes and injecting into a gas chromatograph equipped with a photoionization tube (Photovac 10A10).

Effect of Propylene on In Vitro Growth and Ripening

Fruit were harvested at the green stage (12 days postanthesis; 50% mature) and at the white stage (20 days postanthesis; 71% mature) of development and peduncles trimmed to approximately 55 mm. Fruit fresh weights were matched between treatments. Average fruit weights were 3.11±.50 g for green fruit and 10.96±1.8 g for white fruit. Fruit were placed via peduncles into scintillation vials containing 16 ml of a solution consisting of 88 mM sucrose and 200 $ul.liter^{-1}$ hydroxyquinoline hemi-sulfate (HQS). Four replications were used for each treatment. Each

replication consisted of 4 fruit in 4 vials placed in a 454 ml wide-mouth Mason jar.

Propylene, an analogue of ethylene with approximately 1% of ethylene activity (Burg and Burg, 1967), was employed to determine the ethylene responsiveness of developing strawberry fruit. Air or air and propylene combinations were mixed at the desired concentrations by means of a flow-through system equipped with flow meters and regulating valves (Gull, 1981). A constant flow rate of the different gas treatments was maintained at 15 $ml.min^{-1}$. The propylene gas mixture was administered to the fruit at a concentration of 5000 $ul.liter^{-1}$. The relative humidity within the jars was measured with a psychrometer (Bendix, Model 566) and ranged between 80 and 90%. The air temperature was held at 25^{o} + $3^{o}C$. At intervals, gas samples were withdrawn directly from the outlet tubes with gas-tight syringes. Levels of ethylene and CO_2 were determined as described above. Fruit weights were measured daily. Fruit color was measured at white, pink and red stages with a Hunterlab Colorquest color difference meter.

Effects of ACC and Silver on Fruit Development In Vitro

Green (12 to 14 days postanthesis; 50 to 56% mature) and white fruit (20 days postanthesis; 71% mature) were harvested from the field and placed via peduncle into scintillation vials containing 16 ml of a solution consisting of 88 mM sucrose, 200 $ul.liter^{-1}$ HQS and 0, 1, or 5 mM

1-aminocyclopropane-1-carboxylic acid (ACC). Ethylene production, respiration, fresh weights and color were monitored as described above.

Strawberry fruit harvested 14 days after anthesis (50% mature) were placed in vase solutions via attached peduncles as described above. The vase solutions consisted of 88 mM sucrose and 200 $ul.liter^{-1}$ HQS, with or without 1 mM ACC. Silver thiosulfate (STS) (0.5 mM) was prepared following the method of Reid et al. (1980a). STS (0.5 mM) was added to solutions as a pulse treatment from 0 and 4 days *in vitro*. Fruit were then transferred to solutions without STS for 4 days, then were placed in fresh solutions with 0.25 mM STS from 9 to 12 days *in vitro*. Strawberry fruit were grown in vitro at 27^{o} + 2^{o}C. Ethylene and fresh weights were monitored as described above.

Results

Respiration and Ethylene Production as a Function of Stage of Development

Both respiration and ethylene production of strawberry fruit harvested at selected stages of maturity and held in water to alleviate water stress decreased with advanced maturity (Fig. 4-1). At the green stage, the respiration rate was 240 ml $CO_2 \cdot kg^{-1}\text{-}hr^{-1}$ and this decreased to 55 $ml \cdot kg^{-1}\text{-}hr^{-1}$ for fruit at the green-white stage. Ethylene production was 0.6 $ul \cdot kg^{-1} \cdot hr^{-1}$ at the green stage of development and decreased to less than 0.4 $ul \cdot kg^{-1} \cdot hr^{-1}$ in green-white fruit. Although fresh

weight increased dramatically as fruit changed in color from white to pink, respiration during this period decreased from 40 ml $CO_2 \cdot kg^{-1} \cdot hr^{-1}$ to 20 $ml \cdot kg^{-1} \cdot hr^{-1}$ while ethylene production stabilized near 0.05 $ul \cdot kg^{-1} \cdot hr^{-1}$.

Response of Strawberry Fruit to Exogenous Propylene and ACC

The response to 5000 $ul.liter^{-1}$ propylene of fruit harvested 50 to 70% mature and maintained in vase solutions is shown in Figures 4-2 and 4-3. The responses observed were greatly dependent on fruit maturity. Fruit placed *in vitro* at the white stage failed to exhibit elevated respiration, enhanced fresh weight gain or color formation, over a 7 day period in response to propylene treatment (Fig. 4-2). Endogenous ethylene production during the propylene treatment was too low to register above the 0.1 ppm contaminant ethylene levels in the propylene. Ethylene levels for fruit treated with continuous air remained below 0.5 $ul \cdot kg^{-1} \cdot hr^{-1}$ (data not shown).

Fruit harvested 50% mature (green) and held in vase solutions also failed to exhibit increased respiration when subjected to propylene (Fig. 4-3A). However, unlike white fruit, the fresh weight gains of green fruit were accelerated in response to either intermittent or continuous propylene treatments (Fig. 4-3B). Color development was also enhanced in response to propylene. These effects were related to the length of fruit exposure to propylene. After 4 days *in vitro*, fruit held in atmospheres of continuous (4 days

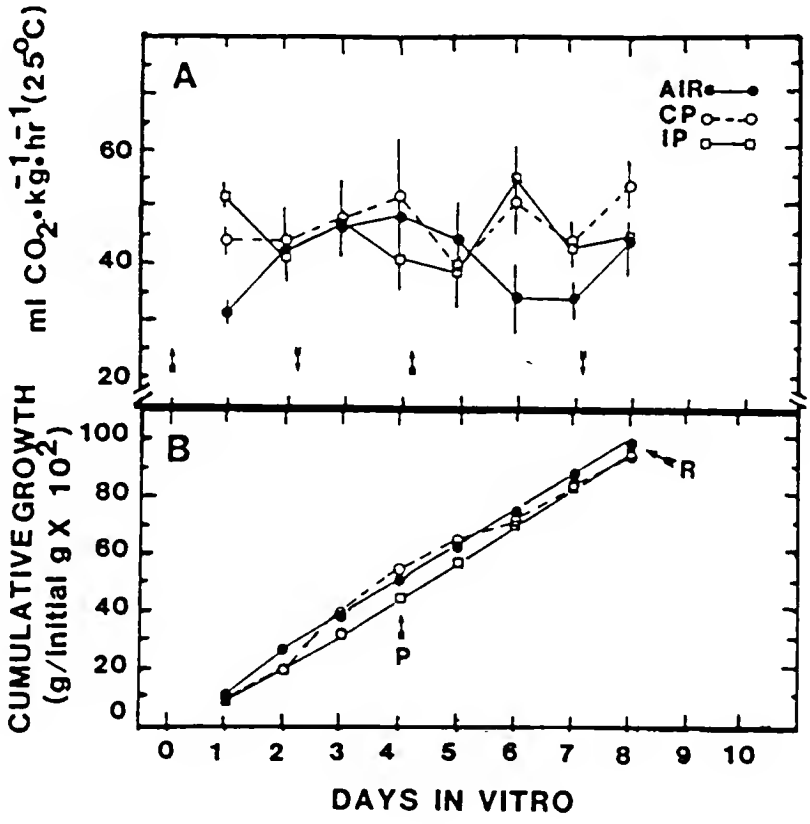

Figure 4-2. Influence of propylene on respiration (A) and weight gain (B) of strawberry fruit harvested 20 days postanthesis (white). Arrows near axis denote times of application and removal of propylene.

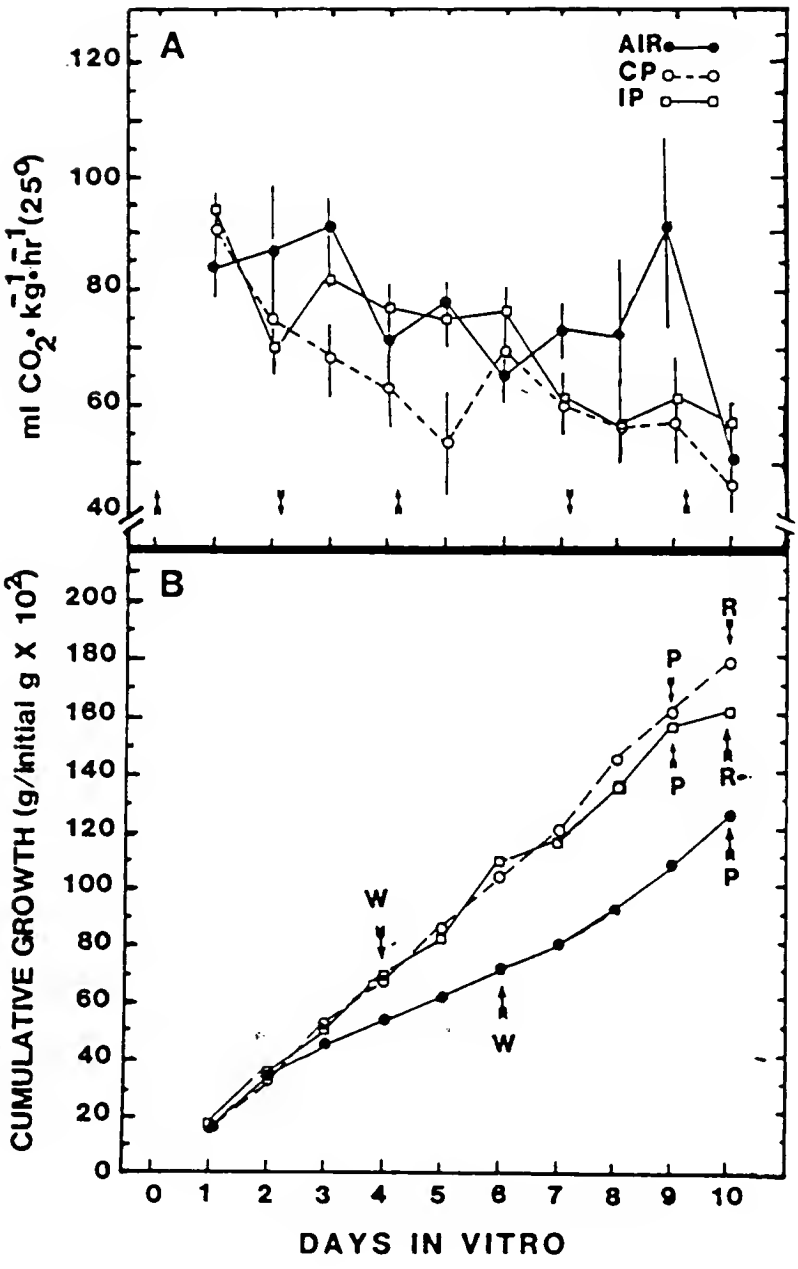

Figure 4-3. Effect of propylene on respiration (A) and weight gain (B) of fruit harvested 12 days postanthesis (green). Arrows near axis denote times of application and removal of propylene.

exposure) or intermittent (2 days exposure) propylene had reached the white stage 2 days earlier and had gained 20% more fresh weight than fruit not exposed to propylene (Fig. 4-3B). Fruit placed *in vitro* at the green stage and continuously exposed to propylene had gained 50% more weight than control fruit after 10 days. Fruit treated intermittently (5 days total) with propylene had gained 30% more fresh weight than control fruit. At this time, fruit initially green and exposed to continuous propylene were red, whereas fruit intermittently exposed were light red and control fruit were pink.

Effect of ACC on Strawberry Fruit Grown In Vitro

As with the propylene treatments, the response of *in vitro* grown strawberry fruit to ACC was greatly dependent on fruit maturity. Ethylene production more than doubled (0.06 $ul.kg^{-1}.hr^{-1}$ to 1 $ul.kg^{-1}.hr^{-1}$) in fruit harvested 70% mature (white) and grown in vase solutions containing 1 mM ACC (Fig. 4-4A). Within 2 days, ethylene production from fruit held in vase solutions containing 1 mM ACC had peaked at a level 30 times higher than ethylene production from fruit without ACC. However, over the entire treatment period, respiration was unaffected by the endogenously produced ethylene (Fig. 4-4B). Gains in fresh weight were the same in both treatments, but color formation was advanced by 2 days in fruit provided with ACC (Fig. 4-4C).

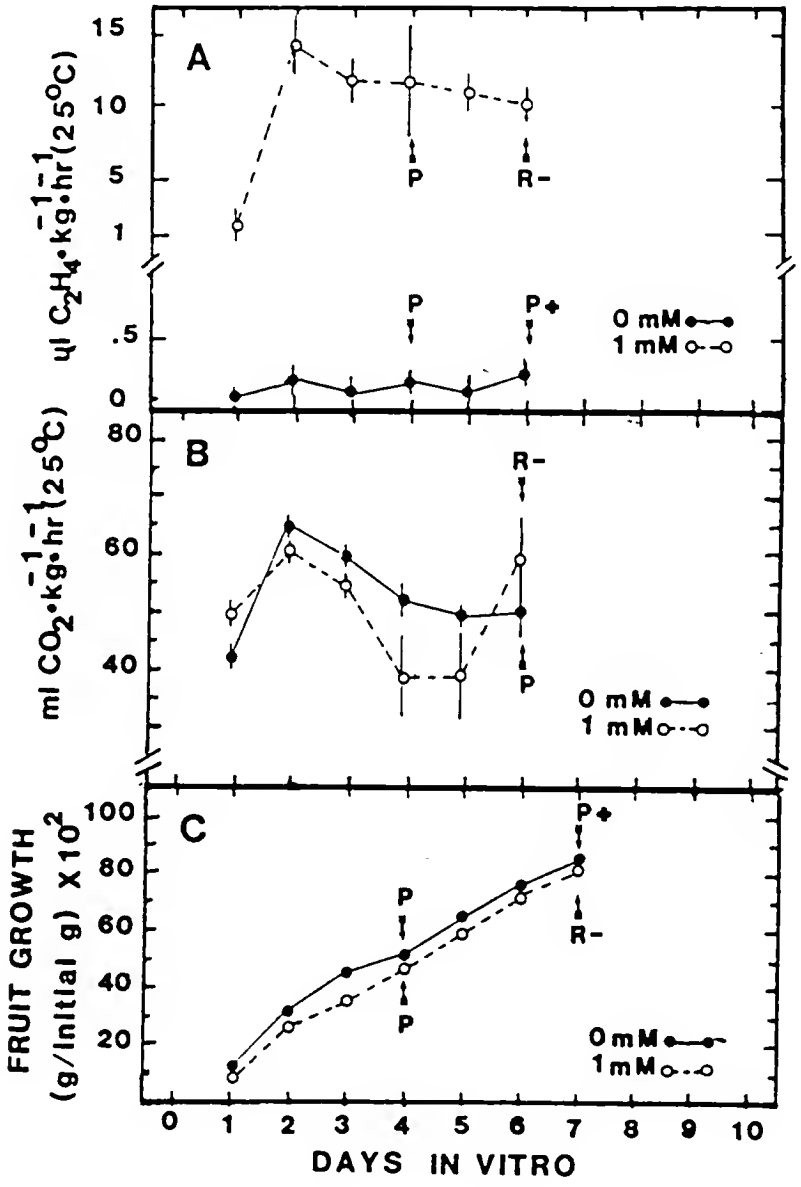

Figure 4-4. Ethylene production (A), respiration (B) and weight gain (C) of fruit harvested at 20 days postanthesis (white) and held in vase solutions with 0 or 1 mM ACC. SE for weight gain are within symbols.

Ethylene production in fruit harvested at 50% maturity (green) and placed *in vitro* with 1 mM ACC increased from 0.5 to 1 $ul.kg^{-1}.hr^{-1}$ within 1 day (Fig. 4-5A). Ethylene production remained at this level until 5 days *in vitro*. From 5 to 7 days *in vitro*, by which time fruit had turned white, ethylene production increased 5-fold from fruit provided with ACC. Fruit held without ACC continued to produce ethylene at a rate of less than 1 $ul \cdot kg^{-1} \cdot hr^{-1}$. The respiration rate of fruit provided with ACC was lower than in the control (Fig. 4-5B). Green fruit held in ACC had gained 30% more in fresh weight after 6 days *in vitro* and 50% more in relative fresh weight after 10 days *in vitro* compared to fruit held in solutions without ACC (Fig. 4-5C). Color changes were advanced by 2 days in fruit provided with continuous ACC relative to the control.

In a subseqent study, green fruit were harvested at a similar stage of development (56% mature) and held in higher concentrations of ACC (5 mM). After 1 day in 5 mM ACC *in vitro*, ethylene production increased 20-fold (0.5 $ul \cdot kg^{-1} \cdot hr^{-1}$ to 10 $ul \cdot kg^{-1} \cdot hr^{-1}$). After 5 days *in vitro*, fruit held in 5 mM ACC had turned white and ethylene production increased to 55 $ul \cdot kg \cdot^{-1} hr^{-1}$ (Fig. 4-6). Fruit held in 1 mM ACC initially produced ethylene at a rate of 2 $ul \cdot kg^{-1} \cdot hr^{-1}$, increasing 10-fold (20 $ul.kg^{-1}\text{-}hr^{-1}$) at 5 days in vitro, by which time fruit had turned white. The ethylene production of the control fruit decreased 40%, from an initial rate of 0.5 $ul \cdot kg^{-1} \cdot hr^{-1}$ to 0.3 $ul \cdot kg^{-1} \cdot hr^{-1}$ after 5 days in vitro.

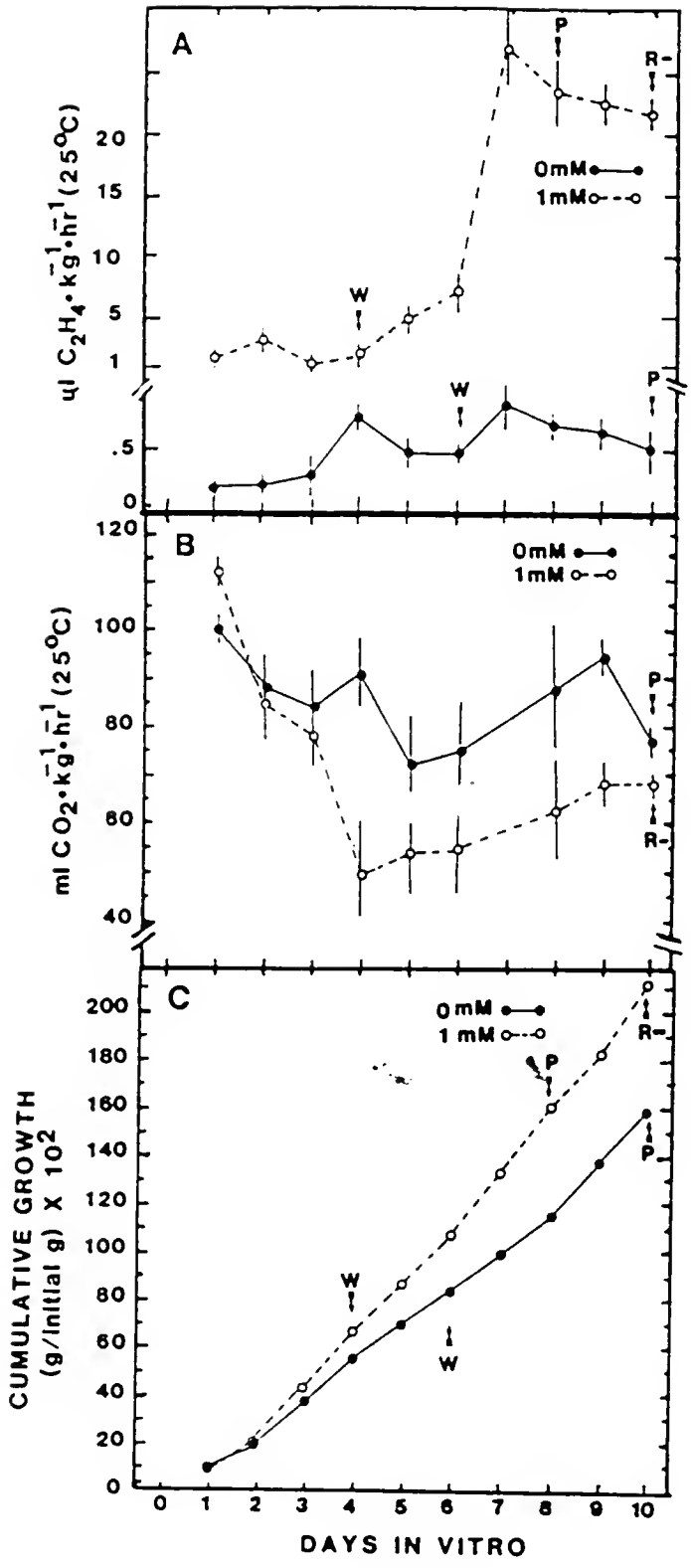

Figure 4-5. The effect of ACC on ethylene production (A) respiration (B) and weight gain (C) of fruit harvested at 14 days post-anthesis (green) stage of development and placed in vase solutions.

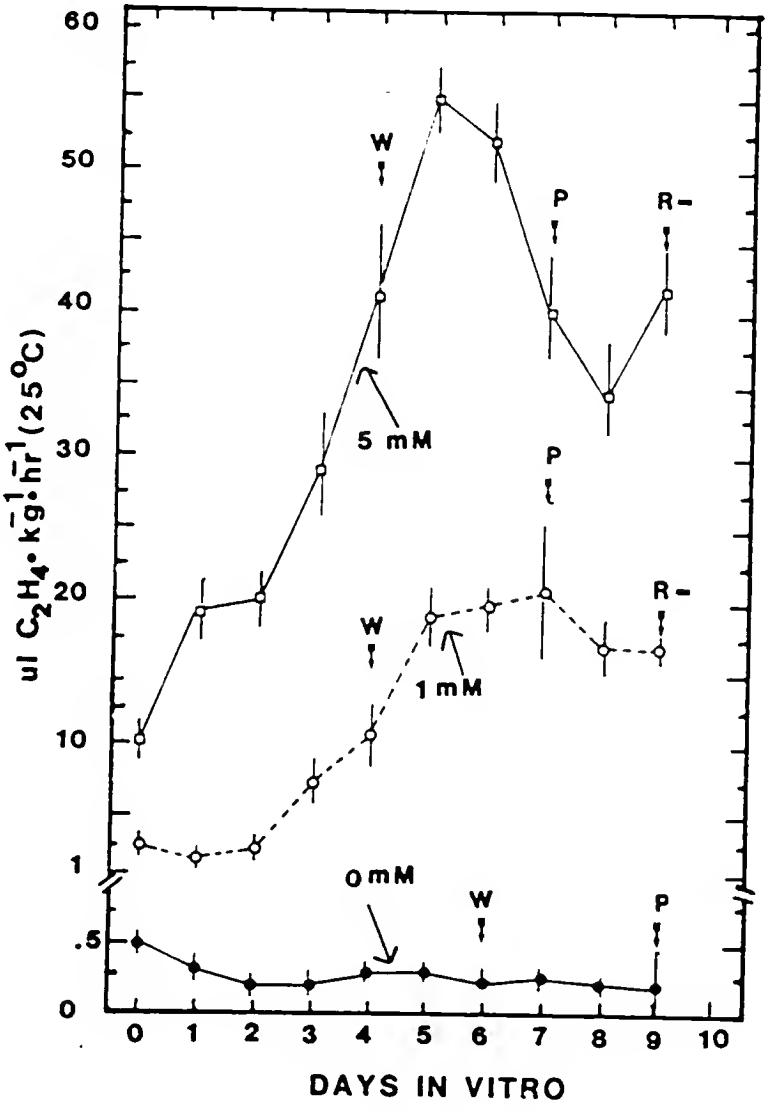

Figure 4-6. Ethylene production from fruit harvested at 14 days post anthesis (green) and placed in vase solutions containing 0, 1,or 5 mM ACC.

Fruit harvested 56% mature (green) and provided with 5 mM ACC *in vitro* exhibited similar respiration rates as fruit grown in 0 or 1 mM ACC (Fig. 4-7A). The cumulative fruit fresh weight gains were 1.70, 1.60 and 1.45 g/initial g for fruit grown in the presence of 5.0, 1.0 and 0 mM ACC, respectively (Fig. 4-7B). Fruit in either the 1 or 5 mM ACC solutions turned white 2 days before the control, and had greater color development after 10 days *in vitro*.

Response of Strawberry Fruit Grown in Solutions Containing Silver

Fruit harvested 50% mature (green) and held in solutions containing 0.5 mM STS, 1 mM ACC or both exhibited increased ethylene production (Fig.4-8A). Ethylene production from fruit held in solutions with STS and without ACC increased 40% over control fruit. After 1 day *in vitro*, fruit held in the STS solutions with ACC exhibited a 100% (1 $ul \cdot kg \cdot hr^{-1}$ to 2 $ul \cdot kg \cdot hr^{-1}$) increase in ethylene production compared to fruit held in ACC solutions without STS, and a 300% increase compared to fruit held without ACC or STS.

Fruit harvested 50% mature (green) and held in solutions containing ACC exhibited enhanced growth after 8 days *in vitro* (Fig. 4-8B). However, fruit held in solutions containing STS exhibited greatly suppressed growth, even in the presence of 1 mM ACC. Fruit in ACC solutions reached the white and pink color stages 1 day earlier than control fruit.

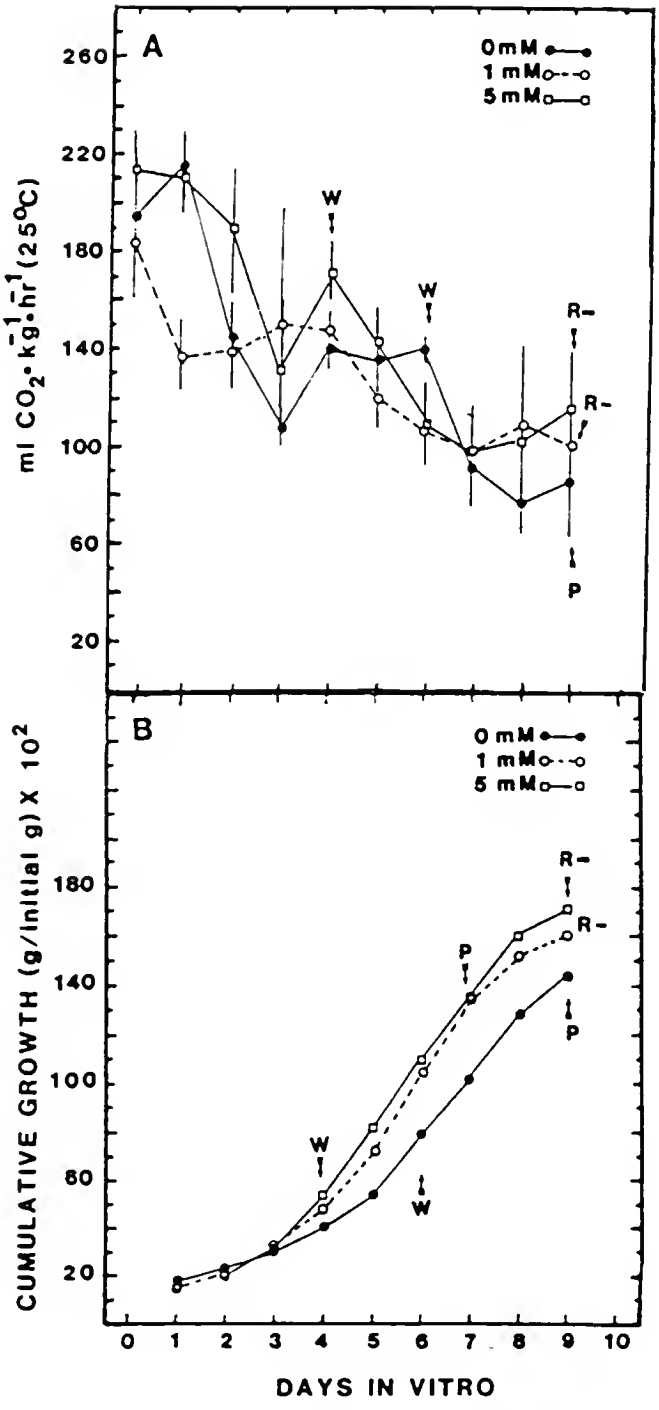

Figure 4-7. Respiration (A) and weight gain (B) of fruit harvested at 14 days post anthesis (green) and placed in vase solutions with 0, 1, or 5 mM ACC. SE bars for weight gain are within symbols.

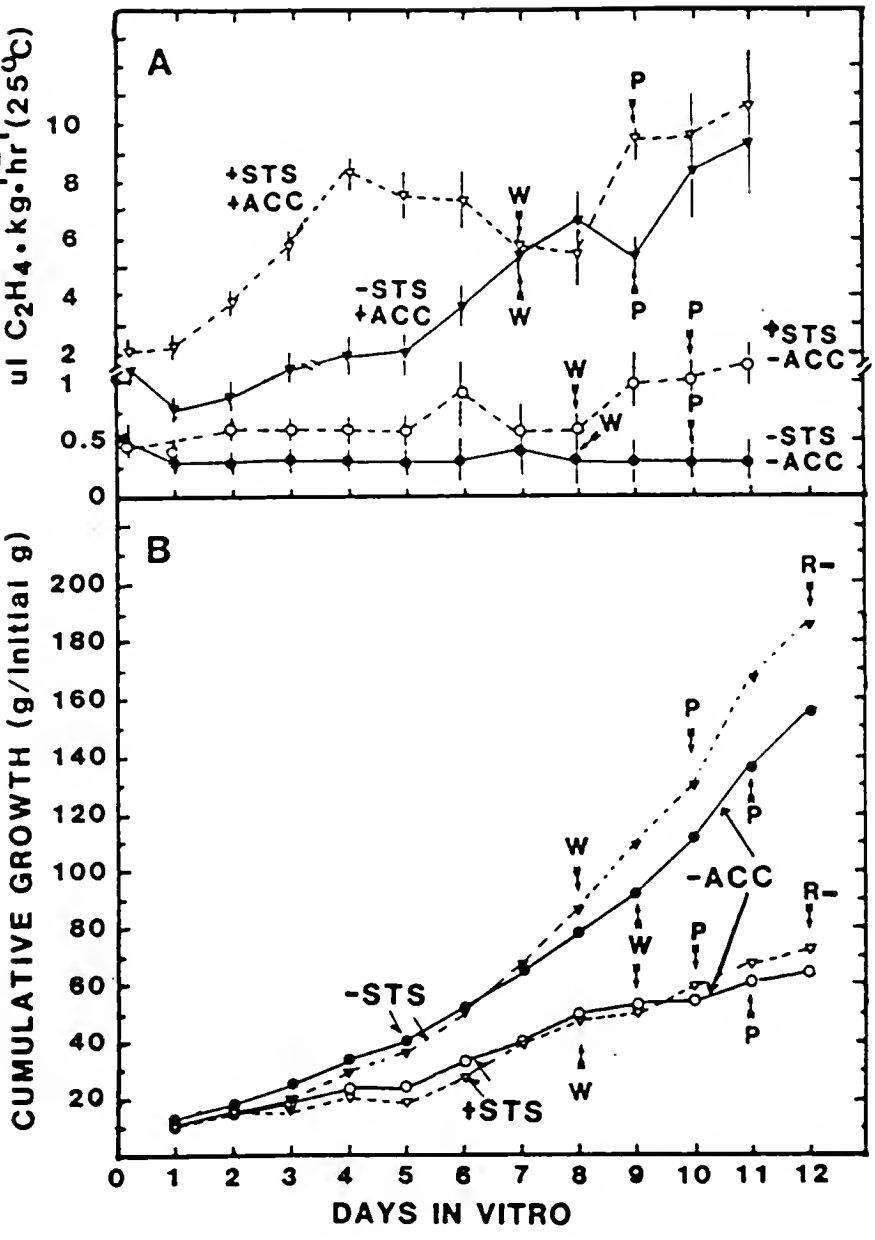

Figure 4-8. The effects of ACC and silver on ethylene production (A), weight gain and color (B) of fruit harvested 14 days post anthesis (green) and placed in vase solutions with with 0 or 1 mM ACC and/or STS.

Although growth was suppressed in the presence of STS, color development was not impaired.

Discussion

The respiration and ethylene production patterns of strawberry fruit harvested at different stages of maturity and maintained and ripened *in vitro* or from fruit harvested from field-grown plants at selected stages during ripening and held in water for 1 day during ripening indicate that this fruit is nonclimacteric. Both respiration and ethylene production rates decreased gradually as fruit ripened. Similar patterns of respiration and ethylene production have been reported for cherry (Blanpied, 1972), carambola (Oslund and Davenport, 1983) and watermelon (Elkashif, 1985) fruit.

The respiration and ethylene production rates from fruit harvested green or white and ripened *in vitro* were higher than those of fruit harvested from the field at selected stages of development. This discrepancy may be due to the effects of sucrose and HQS in the vase solutions used with the *in vitro* fruit. Roses held in vase solutions containing sucrose exhibited higher respiration rates than roses held in vase solutions containing water (Ferreira and De Swardt, 1980). Although the *in vitro* fruit maintained higher levels of ethylene production and respiration, the overall trend was one of decline during ripening, consistent with that from fruit harvested at different stages of development.

Strawberry fruit harvested from 50 to 70% mature and provided with a carbohydrate source failed to exhibit increased respiration rates or stimulation of endogenous ethylene production when continuously or intermittently exposed to ethylene over a 10-day period. Janes et al. (1977) were unable to detect increased respiration in detached, white strawberry fruit exposed to 50 $ul.liter^{-1}$ ethylene for 25 hours. Only one other nonclimacteric fruit type, the cranberry, was reported to exhibit no respiration response when treated with ethylene (Fudge, 1930).

Application of propylene is a widely employed method for investigations of the climacteric nature of fruit (McMurchie et al., 1972). Application of propylene to climacteric fruit, such as banana, results in stimulation of endogenous ethylene production, the onset of ripening and the onset of the respiratory climacteric (McMurchie et al., 1972). Application of propylene to nonclimacteric fruit generally results in enhanced respiration but no stimulation of endogenous ethylene production. The lack of endogenous ethylene production from strawberries in response to propylene is further evidence for the nonclimacteric nature of strawberries. The lack of enhanced respiration obtained with strawberry fruit is in contrast to other nonclimacteric fruit exposed to propylene. Elkashif (1985) found that application of 6500 $ul.liter^{-1}$ propylene to watermelons resulted in a 40% increase in the respiration rates. Saltveit (1977) reported a 38% increase in respiration after

application of 500 ul.liter^{-1} propylene to green bell peppers and McMurchie et al. (1972) reported a 100% increase in respiration of lemons exposed to 500 ul.liter^{-1} propylene.

Growth and color development of strawberry fruit harvested green (50% mature) and maintained *in vitro* was enhanced in the presence of 50 ul.liter^{-1} ethylene. Little stimulation of weight gain or color was evident in fruit harvested white (70% mature) and treated with ethylene. A similar change in sensitivity to ethylene with fruit maturity has been reported for the grape (Hale et al., 1970), a nonclimacteric fruit, and for the fig (Marei and Crane, 1971), a climacteric fruit. Hale et al. (1970) reported that application of 20 ul.liter^{-1} ethylene to grapes on the vine was not effective after the beginning of veraison, but advanced the onset of the second rapid renewed growth phase and ripening if applied just before veraison. Application of 5 ul.liter^{-1} ethylene to figs on the tree between stage II and stage III of growth similarly hastened growth and ripening (Marei and Crane, 1971).

The ability of immature strawberry fruit grown *in vitro* to advance in ripening when exposed to ethylene may be related to the growth pattern of this fruit. Strawberry fruit continue to grow and assimilate sucrose during ripening (Forney and Breen, 1985a), without formation of starch (Knee et al., 1977). The lack of sufficient carbohydrate reserves

may be why Mason and Jarvis (1970) found that strawberries would not soften, increase in soluble solid levels or color normally if detached at the green or white stages of development, despite postharvest treatment with ethylene.

The accelerated growth of the propylene or ethylene treated strawberries may be due to ethylene-enhanced sucrose uptake, as has been reported for other tissues. Saftner (1986) applied ethylene to sugar beet tissue and found a greater uptake of radiolabelled sucrose, which he proposed to be due to increased phloem loading. The loquat, a climacteric fruit, accumulates sucrose within 2 weeks of maturation from other parts of the plant. A rapid increase in the fresh weight occurs during ripening (Hirai, 1980). Hirai (1982) found that ethylene treatment of loquat fruit accelerated color and sugar accumulation. Since sugar accumulation in the loquat is not the result of starch degradation, Hirai (1982) proposed that ethylene triggered an increase in sink activity or changed assimilate distribution patterns. Veen (1985) noted that ACC treatment of carnation buds stimulated the growth of pistils, and the addition of sucrose to the ACC treatments increased pistil growth by an additional 40%.

Suppression of strawberry fruit growth but not ripening in the presence of silver indicates that the processes controlling growth in this fruit are at least partly influenced by ethylene. STS blocked the ethylene-stimulated

growth of pistils in carnation buds (Veen, 1985), and the ripening of banana and tomato tissue slices (Saltveit et al., 1978). However, application of 2 mM STS to cherries, another nonclimacteric fruit, at the yellow-pink stage, failed to prevent ripening (Reid et al., 1985). The ripening of strawberries, defined as color change, held in STS indicates that ripening is independent of ethylene action.

Strawberry fruit were able to assimilate and convert ACC provided in vase solutions into ethylene. A 2-fold increase in ethylene production occurred soon after fruit were placed in the ACC solutions. The peak ethylene production from both white and green fruit was 16 to 30-fold higher than controls. Ethylene production of bell peppers and cucumbers, considered to be nonclimacteric fruit types, was stimulated from basal levels of less than 1 $ul.kg^{-1}hr^{-1}$ to 30 and 9 $ul.kg^{-1}\text{-}hr^{-1}$ by application of 1 mM ACC to excised tissue (Cameron et al. 1979).

The highest levels of ethylene production from ACC occurred after the strawberry fruit turned white but before they reached the pink stage. The association of increased ethylene production with the stage of fruit color development indicates that the activity of ethylene-forming-enzyme (EFE) is related to fruit maturity. Yang et al. (1986) reported that the activity of EFE increased 1000-fold in apple fruit, concurrent with increased ethylene production during ripening.

Conclusions

Strawberry fruit harvested 50 to 70% mature and maintained *in vitro* failed to exhibit a respiratory climacteric or increased ethylene production during normal ripening or in response to exogenously applied propylene. In the presence of ACC, production of endogenous ethylene was greatly stimulated in both green and white fruit, peaking after the initiation of ripening. Respiration did not increase in ACC-treated fruit. Strawberry fruit fresh weight gain and color were enhanced in fruit harvested green and maintained *in vitro* in the presence of ethylene but only fresh weight gain was inhibited by STS treatment. The respiration and ethylene production of fruit harvested at green through red stages of development decreased with advanced maturity, similar to the decline exhibited by fruit ripened *in vitro*. On the basis of these results, the strawberry can be classified as a nonclimacteric fruit. Additionally, ethylene appears to be involved in the fresh weight gain of strawberry fruit.

CHAPTER V
REGULATION OF ETHYLENE PRODUCTION IN STRAWBERRY FRUIT

Historically, the ripening pattern of fleshy fruit has been categorized as climacteric or nonclimacteric on the basis of respiration patterns during maturation and ripening (Biale and Young, 1981). Additionally, McMurchie et al. (1972) showed that there is a difference in the ethylene production capacity between climacteric and nonclimacteric fruit. The climacteric fruit undergo a distinct rise in respiration and ethylene emanation, whereas nonclimacteric fruit do not. In climacteric fruit, the increase in ethylene production is temporally associated with a number of ripening changes, including softening, pigment synthesis and/or chlorophyll degradation. Ethylene has been thought to function as a hormone, initiating and coordinating ripening events in climacteric fruits (Rhodes, 1980). In the nonclimacteric fruits studied, such as lemon (McMurchie et al., 1972), cherry (Reid et al., 1985), carambola (Oslund and Davenport, 1983) and watermelon (Elkashif, 1985), ripening was not found to be associated with increased ethylene production. Since nonclimacteric fruit display no increase in ethylene production, the involvement of ethylene, if any, as a ripening initiator in these fruits must relate to an increased tissue sensitivity to ethylene (McGlasson, 1978).

The intermediates involved in the formation of ethylene have been identified as methionine, S-adenosylmethionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC) (Adams and Yang, 1979). This pathway of ethylene biosynthesis has been characterized as follows:

Methionine----SAM---ACC---ethylene

The pathway of ethylene synthesis has been found without exception in all higher plant systems thus far studied (Yang and Hoffman, 1984). Studies of the regulation of ethylene synthesis in climacteric fruit have shown that the major controlling enzyme is ACC synthase, which converts SAM to ACC (Yang, 1987). Ethylene forming enzyme (EFE), which converts ACC to ethylene, is constitutive but its activity can be enhanced by the application of ethylene or ACC. The degree of enhancement is dependent on fruit maturity (Hoffman and Yang, 1982). Application of ACC to cucumber, pepper or squash, all nonclimacteric fruits, resulted in a 10 to 30-fold stimulation of ethylene production (Cameron et al., 1979). In citrus, one of the most intensely studied nonclimateric fruit, investigations have focused on the wound response of citrus (Hyodo and Nishino, 1981; Riov and Yang, 1982) rather than regulation during fruit development and ripening.

Silver, applied as silver thiosulfate (STS), has been shown to be a potent inhibitor of ethylene action. Silver

thiosulfate inhibited the wilting of carnation flowers (Reid et al., 1980), blocked the elongation of carnation pistils in the presence of ACC (Veen, 1985) and inhibited lycopene synthesis and polygalacturonase activity in tomato fruit (Hobson et al., 1984). However, the effects of STS on nonclimacteric fruit ripening are unknown.

There is a lack of information regarding the regulatory features of ethylene in nonclimacteric fruit. Previously, the use of an *in vitro* system allowing the normal development of strawberry fruit demonstrated clearly that these fruit exhibit nonclimacteric ripening behavior (Chapter IV). The objectives of this study were to determine the levels of ACC and the activities of ACC synthase and EFE during ripening. Additionally, the effects of exogenously applied silver thiosulfate (STS), a known inhibitor of ethylene action, and ACC on ethylene biosynthesis were examined.

Materials and Methods

Plant Material

'Pajaro' strawberry (*Fragaria* X *annassa* Duch.) fruit were tagged at anthesis and harvested from field plantings in Gainesville and Dover, Florida. Fruit used for measuring ethylene production capacities in detached fruit were harvested at the green, white, pink and red stages of color development with attached peduncles, placed in plastic bags and transported on ice to the laboratory. Fruit to be developed *in vitro* were harvested at the green stage (50%

mature) with intact peduncles, placed in plastic bags and transported on ice to the laboratory. The maturity of green fruit was calculated as the percent of days from anthesis to field ripeness. Fruit not used immediately were stored at $1^{o}C$ and used within 1 week.

Treatment of Strawberry Fruit with STS or ACC

Strawberry fruit were harvested at the green stage (50% mature) with peduncles attached, weighed and placed individually via peduncle into 16 ml scintillation vials containing 15 ml of vase solution (88 mM sucrose and 200 $ul.liter^{-1}$ hydroxyquinoline sulfate (HQS)), with or without 1 mM ACC. Silver thiosulfate (STS) (0.5 mM) was prepared following the method of Reid et al. (1980) and used in the vase solutions from 0 to 4 days. Fruit were then transferred to solutions without STS for 4 days before being returned to fresh solutions with 0.25 mM STS for 3 additional days. *In vitro* grown fruit were maintained at $27^{o}+2^{o}C$. Four fruit per treatment were sacrificed after 4 days *in vitro* for measuring ACC content, EFE and ACC synthase activities. The remaining fruit were sacrificed when the fruit were ripe (red). Determinations of ethylene production, ACC and assays for EFE and ACC synthase were performed as described below.

Comparison of Ethylene Production Between Receptacle and Calyx

Green strawberry fruit were harvested, weighed, and placed in vase solutions containing 1 mM ACC as described above. After 1 day *in vitro*, fruit were weighed and calyxes

excised. Ethylene measurements were taken from fruit and calyxes at intervals from 1 to 6 hours and again after 24 hours as described below. After 2 days *in vitro*, calyxes were removed from the remaining fruit and the ACC content and EFE activity of fruit and calyxes were determined as described below.

Ethylene measurements. Four replications, consisting of 4 detached fruit with peduncle per color stage or 4 fruit grown *in vitro*, with calyxes excised, were placed separately in 16 ml vials in 252 ml jars. Excised calyxes were placed in 25 ml Erlenmeyer flasks. Jars containing fruit and flasks containing calyxes were sealed for 1 and 0.5 hours, respectively, at $25^{o}+2^{o}$ C. Afterwards, 0.5 ml of atmosphere was withdrawn using gas-tight syringes and analyzed using a gas chromatograph equipped with a photoionization detector (Photovac 10A10) and an activated alumina column. For fruit grown *in vitro* with ACC and/or STS, 4 replications consisting of 4 fruit in individual 16 ml vials per 454 ml jar were maintained in a flow-through system with a constant flow rate of 15 $ml.min^{-1}$ air. The relative humidity within the jars ranged between 80 and 90%, and the air temperature was held at $25^{o}+2C$. At appropriate intervals, gas samples were withdrawn directly from the outlet tubes with gas-tight syringes and levels of ethylene were determined as described above.

Assay of EFE activity. EFE activity was determined by measuring the capacity of the strawberry tissue to convert excess exogenously supplied ACC to ethylene (Hoffman and Yang, 1982). Tissue plugs (8.5 mm in diameter) were taken from freshly harvested fruit by insertion of a sterile, number 5 cork borer through the center of the fruit, bisecting the cortex, pith and epidermal layers. The remainder of the receptacle was frozen at -30°C for the ACC assay (see below). Each plug from green or *in vitro* grown fruit was sliced into discs 3 mm thick, to aid solution infiltration. One plug per fruit, intact or sliced into discs, was vacuum infiltrated for 2 minutes with a solution of 2% (w/v) KCl containing 2.5 to 5 mM ACC. Immediately after infiltration, tissue plugs from white, pink or red fruit were placed in 25 ml Erlenmeyer flasks and sealed with serum caps for 1 hour at 25°C. Discs from fruit harvested green or grown *in vitro* were placed in 50 ml Erlenmeyer flasks after infiltration, incubated for 3 hours, then sealed with serum caps for 1 hour. The accumulated ethylene concentration in the head space was determined as described above.

Determination of ACC. Fruit used for EFE assays were frozen at -30° overnight. Five g of frozen receptacle tissue or 1 g of frozen calyx tissue was then homogenized in 4 ml/g cold 80% ETOH with a Sorvall Omnimixer at maximum speed for 2 minutes. Homogenates were held on ice for 8 hours, then centrifuged at 10,000 G for 20 minutes. ACC in

the supernatant was assayed directly by the method of Lizada and Yang (1979), omitting the ion-exchange purification. The efficiency of conversion of ACC to ethylene ranged from 70 to 90%.

Assay of ACC synthase. Strawberry fruit tissue was extracted and ACC synthase assayed according to Yu et al. (1979) except that the concentration of pyridoxal phosphate was increased to 10 uM in the extraction buffer and to 5 uM in the dialysis buffer. Procedures were performed in a cold room at 2°C. Ten g of frozen receptacle tissue was homogenized in 20 ml of cold 100 mM Epps (pH 8.5) with a mortar and pestle, then the homogenate was squeezed through 4 layers of cheesecloth. The filtrate was centrifuged at 20,000 G at 2°C for 20 minutes. The supernatant was dialyzed 16 hours against 10 volumes and 3 changes of 10 mM Epps (pH 8.5).

Results

Changes in ACC Content and Activities of EFE and ACC Synthase from Strawberry Fruit Detached at Different Stages of Color Development

The ACC contents in strawberry fruit harvested from the field at pink and red stages were 30% higher than those in green or white fruit (Table 5-1). The activity of EFE was 49% lower in white fruit compared to green fruit, but thereafter changed little as the fruit ripened. Ethylene production decreased dramatically as fruit matured from green to white, then remained constant from white through red stages. There was a temporal relationship between EFE

Table 5-1. Comparison of ACC content, EFE activity and ethylene production from strawberry fruit harvested from the field at different color stages.

Color Stage	Fruit Weight	ACC Content	EFE Activity	Ethylene
	(g)	(nmol/g)	(nmol/g-hr)	(nmol/g-hr)
Green	2.59	1.24	0.089	0.0231
White	6.10	1.19	0.045	0.0016
Pink	15.19	1.93	0.045	0.0015
Red	15.67	1.95	0.031	0.0014
LSD[z] (0.05)	0.50	0.36	0.009	0.0071

[z]LSD=least squares difference of means from 4 replications.

activity and ethylene production. However, ethylene production decreased 93% in white fruit compared to green fruit, whereas EFE activity decreased only 49%. No ACC synthase activity could be detected at any stage of maturation. The EFE activity was 2 to 4-fold higher than ethylene production throughout development. The concentration of ACC was significantly greater at the pink and red stages than at the green or white stages.

Response of In Vitro Grown Fruit to Exogenously Applied ACC and STS

After 4 days *in vitro*, the ACC content, EFE activity and ethylene production in green strawberry fruit provided with silver (STS) increased (Table 5-2), presumably due to enhanced ACC synthase activity resulting from silver-induced wounding. An increase in ACC content, ethylene production, and EFE activity resulted from incubation in ACC. The treatment combination of ACC and STS resulted in levels of ACC substantially higher than those resulting from STS or ACC alone. However, no ACC synthase could be detected in fruit from this or the other treatments after 4 days *in vitro*. The activity of EFE was enhanced 3-fold in fruit treated with STS alone, almost 5-fold in fruit treated with ACC for 4 days and 15-fold in fruit treated with both ACC and STS. Ethylene production increased 75% in fruit treated with STS, 456% in fruit treated with ACC and 2000% in fruit treated with both STS and ACC.

Table 5-2. Effect of STS and ACC addition to vase solutions with strawberry fruit on ethylene biosynthesis of fruit grown *in vitro*.

Treatment	Days In Vitro					
	Day 4			Day 11		
	ACC	EFE	C_2H_4	ACC	EFE	C_2H_4
	(nmol/g)	(nmol/g-hr)	(nmol/g-hr)	(nmol/g)	(nmol/g-hr)	(nmol/g-hr)
-STS -ACC	2.72	0.123	0.016	3.42	0.126	0.013
+STS -ACC	3.61	0.149	0.028	3.80	0.245	0.049
-STS +ACC	26.20	0.209	0.089	104.00	0.983	0.375
+STS +ACC	118.70	0.779	0.335	94.80	0.989	0.424
LSD (0.05)	0.31	0.010	0.005	0.26	0.036	0.018

All fruit harvested green and held 11 days *in vitro* had developed to the red stage. Ethylene production, activity of EFE and content of ACC changed little in fruit held without ACC or STS (Table 5-2). At this time, EFE activity doubled or increased 8-fold in fruit provided with ACC or ACC and silver, respectively. The ethylene production from treated fruit increased 3-fold with silver and 30-fold with ACC or ACC and silver, relative to the control fruit.

Contributions of Calyx and Receptacle Tissues to Ethylene Production

The ACC content of receptacle tissue from green fruit was greater than that of calyx tissue from green fruit held for 2 days in vase solutions (Table 5-3). The activity of EFE in calyx tissue was markedly higher than that present in receptacle tissue. Ethylene production in calyxes excised (wounded) from fruit held without ACC was about the same as in intact fruit (Fig. 5-1). Since the ethylene production from fruit with calyxes compared to that from fruit without calyxes was similar, the contribution of the calyx to the ethylene production from intact fruit was probably inconsequential (Fig. 5-1).

Strawberries held *in vitro* with ACC had enhanced EFE activity in both the receptacle and calyx tissue (Table 5-3). The activity of EFE in ACC-treated receptacle tissue was 12-fold higher after 2 days, whereas the activity of EFE in the ACC-treated calyx tissue was 4- to 6-fold higher, compared to the control. Ethylene production from fruit treated with ACC was slightly greater in fruit with intact

Table 5-3. Comparison of ACC content, EFE activity and ethylene production from green strawberry fruit receptacle tissue and calyx tissue after incubation in 1 mM ACC in vase solutions for 2 days.

Treatment	Tissue	Fresh Weight	ACC Content	EFE Activity
		(g)	(nmol/g)	(nmol/g-hr)
-ACC	Fruit	4.51	3.02±.31	0.096±0.010
	Calyx	1.04	1.33±.21	48.101±2.10
+ACC	Fruit	4.96	23.98±1.63	0.366±0.019
	Calyx	1.00	5.23±0.91	80.512±4.83

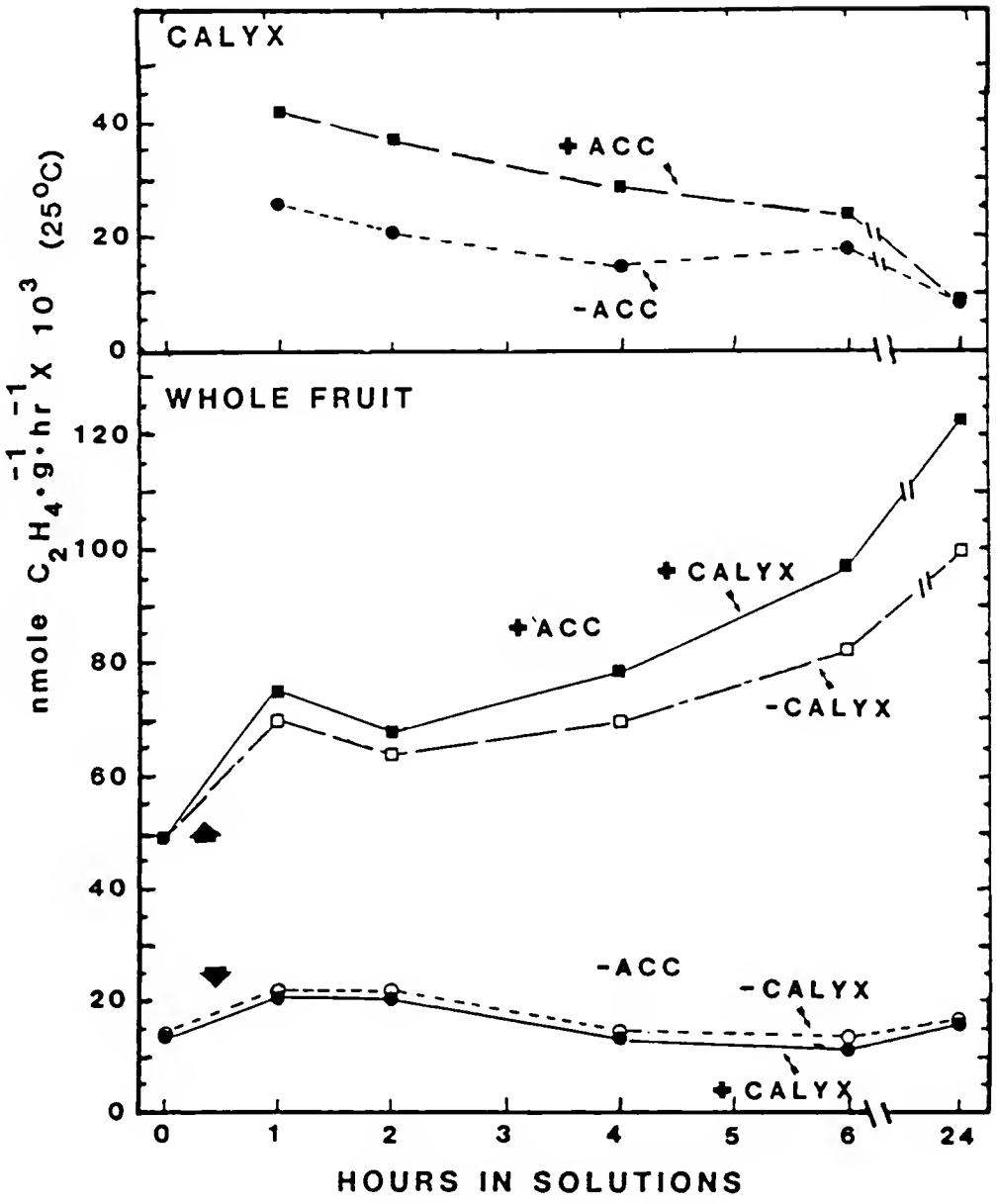

Figure 5-1. Ethylene production from calyxes and whole fruit harvested at the green stage after calyx removal and treatment with 1 mM ACC. Large arrows indicate time of calyx excision.

calyxes than from fruit without calyxes (Figure 5-1). Calyxes excised from fruit previously treated with ACC exhibited a 40% increase in ethylene initially compared to calyxes excised from untreated fruit but ethylene production declined to control levels between 6 and 24 hours after excision.

Discussion

The ethylene production rate and EFE activity in strawberry fruit decreased sharply as fruit matured from green to white, but little change occurred with further fruit development. The ACC content was low and increased only slightly, but significantly, as fruit developed from the green to red stage. The results with strawberry, a nonclimacteric fruit, contrast with the reported increases in EFE activity, ACC content and ethylene production in ripening climacteric fruit (Apelbaum et al., 1981; Brecht and Kader, 1984b; Sitrit et al., 1986).

The drop in ethylene production during ripening could be due to decreased ACC synthase activity, EFE impairment or a compartmentalization of ACC. Loss in ethylene production as a function of decreased ACC synthase has been reported in postclimacteric avocados (Sitrit et al., 1986), apples (Yang et al., 1986), and tomatoes (Kende and Boller, 1981). Although no ACC synthase activity was found, the activity of EFE decreased in ripening strawberry fruit, indicating either a loss of EFE activity or a decrease in the availability of

ACC to EFE. Brecht and Kader (1984b) suggested that the increased levels of ACC in ripening nectarines may have been due to the inefficiency of transport between the site of ACC formation and the site of utilization, since EFE activity remained higher than ethylene production rates.

In vitro application of ACC induced a considerable increase in EFE activity and ethylene production from strawberry fruit receptacle and calyx tissues. Both the receptacle and calyx tissues accumulated ACC. The enhancement by ACC of ethylene production in strawberries is similar to reports for excised tissues from the nonclimacteric bell pepper and squash fruit (Cameron et al., 1979). The EFE activity increased in ripening, nonclimacteric strawberry fruit in response to applied ACC. Increased EFE activity has been found in ripening climacteric avocados (Blumenfield et al., 1986), cantaloupe (Hoffman and Yang, 1982), and apples (Yang et al., 1986).

The application of STS to in vitro strawberry fruit resulted in a higher ACC content even though EFE activtiy was also higher, indicating enhanced ACC synthase activity. However, no ACC synthase could be detected from fruit treated with ACC and/or silver, or from fruit field-grown and sampled throughout ripening. The methods used to isolate ACC synthase from strawberry fruit resulted in successful isolation of ACC synthase from tomato fruit (data not shown). The ACC synthase enzyme has been reported to be very labile

in tomato fruit (Acaster and Kende, 1983). Nakajima and Imaseki (1986) reported that the ACC synthase purified from wounded winter squash (Cucrubita maxima Duch.) mesocarp was composed of 2 polypeptide subunits of 84000 molecular weight, in contrast to a report of 55000 molecular weight ACC synthase from tomato fruit (Acaster and Kende, 1983). Possibly, in strawberry fruit, the enzyme has a different structure, rendering it more labile than ACC synthase from other plant tissues. Additionally, strawberry fruit have low amounts of protein (Huber, 1984) which may be subjected to proteases.

The individual capacities of strawberry fruit receptacle and calyx tissues to produce ethylene were similar, although the EFE in calyx tissue was much greater than in receptacle tissue. Fruit with calyxes excised produced the same amount of ethylene than intact fruit, indicating that ethylene production in strawberry fruit is primarily from receptacle tissue.

Conclusions

The ACC content of strawberry fruit changed little during strawberry fruit ripening, although EFE activity and ethylene production decreased when fruit matured from the green to the white stage. The role of ACC synthase in this process needs further study.

CHAPTER VI
SUMMARY AND CONCLUSIONS

Unlike many fruit, green strawberry fruit fail to ripen normally once detached from the plant. Previous researchers have shown that strawberry fruit continue to grow during ripening, and that detached fruit fail to advance in ripening if subjected to postharvest applications of ethylene. The purpose of this work was to develop a system whereby strawberry fruit could be harvested from the field and ripened under controlled conditions. This system allowed manipulation of ripening conditions as well as controlled measurements of ethylene production, respiration and growth during the ripening processes.

Strawberry fruit were detached with intact peduncles at the immature-green stage and successfully ripened by insertion of the peduncles into vase solutions consisting of 200 $ul.liter^{-1}$ hydroxyquinoline sulfate (HQS) and 88 mM sucrose. Fruit as young as 25% mature were successfully ripened in this *in vitro* system. Fruit harvested 70% mature (white stage), however, did not require the presence of sucrose to gain weight or develop color. Of the 2 cultivars tested, 'Pajaro' fruit responded more favorably to the *in vitro* system than 'Douglas' fruit.

Fruit held *in vitro* were treated with exogenous propylene or 1-aminocyclopropane-1-carboxylic acid (ACC) to determine the effects of ethylene on strawberry fruit ripening, respiration and ethylene production. Fruit harvested 50% mature, at the green stage, exhibited accelerated development of color and weight gain in response to the ethylene, but respiration was not stimulated. Fruit harvested 70% mature, at the white stage, did not respond to the ethylene. Treatment of fruit with silver, a known inhibitor of ethylene action, did not delay fruit ripening but suppressed fruit growth, both in the presence and absence of ACC. Respiration and ethylene production were highest in green fruit then decreased as fruit ripened. The downward drift in respiration and ethylene production during fruit ripening, and the absence of autocatalytic/system II endogenous ethylene production upon exposure to propylene provide evidence for the nonclimacteric classification of strawberry fruit.

Assays of strawberry fruit tissue for ACC, the immediate precursor of ethylene, revealed little change in ACC levels throughout fruit development. The activity of ethylene-forming enzyme (EFE), which converts ACC to ethylene, was highest in green fruit, and decreased 50% in white fruit. The lower EFE activity was maintained throughout ripening. Changes in EFE activity during fruit development were temporally related to ethylene production.

No ACC synthase could be detected in fruit at any stage of maturity.

In conclusion, strawberry fruit, harvested at a stage of development well in advance of ripeness, could be successfully ripened *in vitro* by employing a vase solution containing a carbohydrate source and a biocide. Measurements of ethylene production and respiration made during strawberry fruit growth demonstrated no rise in respiration or ethylene production during ripening. Application of exogenous propylene, although advancing color development and weight gain of *in vitro* fruit, did not stimulate endogenous ethylene production. Based on these results, the strawberry fruit can be conclusively classified as nonclimacteric. These studies show for the first time that no large increase in ACC content or EFE activity occurs during strawberry fruit development. The enhanced fruit growth and color development exhibited by strawberry fruit maintained *in vitro* in the presence of ethylene may indicate involvement of ethylene in sucrose uptake or the location of ethylene receptors on both tonoplast and plasmamembrane. Further work remains to be done to determine the controlling factors in the ethylene biosynthesis of strawberry fruit, and to identify the controlling mechanisms of strawberry fruit ripening.

LIST OF REFERENCES

Abbott, A.J., G.R. Best and R.A. Webb. 1970. The relation of achene number to berry weight in strawberry fruit. J. Hort. Sci. 45:215-222.

Abeles, F.B. 1973. Ethylene in Plant Biology, pp. 206-208. Academic Press, New York.

Abeles, F.B. and S.G. Wydowski. 1987. Inhibitors of ethylene synthesis and action: A comparison of their activities in a lettuce root growth model system. J. Amer. Soc. Hort. Sci. 112:122-125.

Acaster, M.A. and H. Kende. 1983. Properties and partial purification of 1-aminocyclopropane-1-carboxylate synthase. Plant Physiol. 72:139-145.

Adams, D.O. and S.F. Yang. 1979. Ethylene biosynthesis: Identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc. Natl. Acad. Sci. 76:170-174.

Akamine, E.K. and T. Goo. 1978. Respiration and ethylene production in mammee apple (Mammea americana L.). J. Amer. Soc. Hort. Sci. 103:308-310.

_____. 1979. Respiration and ethylene production in fruits of species and cultivars of Psidium and species of Eugenia. J. Amer. Soc. Hort. Sci. 104:632-635.

Antoszewski, R. 1973. Translocation of IAA in the strawberry plant in relation to the accumulation of nutrients in the fruit. Acta Hort. 34:85-88.

_____, and E. Lis. 1968. Translocation of some radioactive compounds from the strawberry receptacle to the mother plant. Bull. Acad. of Fruit Sciences. 16:443-446.

Apelbaum, A., A.C. Burgoon, J.D. Anderson, T. Solomos and M. Lieberman. 1981. Some characteristics of the system converting 1-amino-cyclopropane-1-carboxylic acid to ethylene. Plant Physiol. 67:80-84.

Archbold, D.D. and F.G. Dennis, Jr. 1984. Quantification of free ABA and free and conjugated IAA in strawberry achene and receptacle tissue during fruit development. J. Amer. Soc. Hort. Sci. 109:330-335.

_____. 1985. Strawberry receptacle growth and endogenous IAA content as affected by growth regulator application and achene removal. J. Amer. Soc. Hort. Sci. 110:816-820.

Atta-Aly, M.A., M.E. Saltveit, and G.E. Hobson. 1987. Effect of silver ions on ethylene biosynthesis by tomato fruit tissue. Plant Physiol. 83:44-48.

Austin, M.E., V.G. Shutak and E.P. Christopher. 1960. Color changes in harvested strawberry fruits. Proc. Amer. Soc. Hort. Sci. 75:382-386.

Avigdori-Avidov, H. 1986. Strawberry, pp.419-448. In: S.P. Monselise (Ed.), CRC Handbook of Fruit Set and Development. CRC Press, Boca Raton, FL.

Bajaj, Y.P. and W.B. Collins. 1968. Some factors affecting the in vitro development of strawberry fruits. Proc. Amer. Soc. for Hort. Sci. 93:326-333.

Barnes, M.F. and B.J. Patchett. 1976. Cell wall degrading enzymes and the softening of senescent strawberry fruit. J. Food Science 41:1392-1395.

Beyer, E. 1976. A potent inhibitor of ethylene action in plants. Plant Physiol. 58:268-271.

Biale, J.B. and R.E. Young. 1981. Respiration and ripening in fruits-retrospect and prospect, pp. 1-40. In: J. Friend and M.J. Rhodes (Eds.), Recent Advances in the Biochemistry of Fruits and Vegetables. Academic Press, New York.

Blanpied, G.D. 1972. A study of ethylene in apple, red raspberry, and cherry. Plant Physiol. 49:627-630.

Blumenfeld, A., Y. Sitrit and J. Riov. 1986. Avocado fruit ripening and ethylene biosynthesis. Acta Hort. 179:787-791.

Bollard, E.G. 1970. The physiology and nutrition of developing fruits, pp.387-485. In: A.C. Hulme (Ed.), The biochemistry of fruits and their products. Vol. I Academic Press, New York.

Boller, T., R.C. Herner and H. Kende. 1979. Assay for and enzymatic formation of an ethylene precursor, 1-aminocyclo-propane-1-carboxylic acid. Planta 145:293-303.

Brady, C.J. 1987. Fruit ripening. Ann. Rev. Plant Physiol. 38:155-178.

Brecht, J.K., and A.A. Kader. 1984a. Ethylene production by 'Flamekist' nectarines as influenced by exposure to ethylene and propylene. J. Amer. Soc. Hort. Sci. 109:302-305.

_____. 1984b. Regulation of ethylene production by ripening nectarine fruit as influenced by ethylene and low temperature. J. Amer. Soc. Hort. Sci. 109:869-872.

Burdett, A.N. 1970. The cause of bent neck in cut roses. J. Amer. Soc. Hort. Sci. 95:427-431.

Burg, S.P. and E.A. Burg. 1965. Relationship between ethylene production and ripening in bananas. Botan. Gaz. 126:200-204.

_____. 1967. Molecular requirements for the biological activity of ethylene. Plant Physiol. 42:144-152.

Burroughs, L.F. 1960. The free amino-acids of certain British fruits. J. Sci. Food Agric. 11:14-18.

Cameron, A.C., C.A.L. Fenton, Y. Yu, D.O. Adams and S.F. Yang. 1979. Increased production of ethylene by plant tissues treated with 1-aminocyclopropane-1-carboxylic acid. HortScience 14:178-180.

Coombe, B.G. 1976. The development of fleshy fruits. Ann. Rev. Plant Physiol. 27:507-528.

Crane, J.C. and R.E. Baker. 1953. Growth comparisons of the fruits and fruitlets of figs and strawberries. Proc. Amer. Soc. Hort. Sci. 62:257-260.

Creasy, E.C., E.C. Maxie, and V.L. Singleton. 1965. Characterization of flavanoids in Fragaria. Proc. Amer. Soc. Hort. Sci. 85:325-331.

Culpepper, C.W., J.S. Cladwell, and H.H. Moon. 1935. A physiological study of development and ripening in the strawberry. J. Agric. Res. 50:645-696.

Dana, M.N. 1980. The strawberry plant and its environment, pp. 33-44. In: N.F. Childers (Ed.), The Strawberry. National Strawberry Conference.

Dayawon, M.M. and V.G. Shutak. 1967. Influence of N6-Benzyladenine on the postharvest rate of respiration of strawberries. HortScience 2:12.

Den Nijs, A.P.M. and D.L. Visser. 1980. Induction of male flowering in gynoecious cucumbers by silver ions. Euphytica 29:273-280.

Dennis, F.G., J. Lipecki, and C.L. Kiang. 1970. Effects of photoperiod and other factors upon flowering and runner development of three strawberry cultivars. J. Amer. Soc. Hort. Sci. 95:750-754.

Duewer, R.G. and C.C. Zych. 1967. Heritability of soluble solids and acids in progenies of the cultivated strawberry (Fragaria X Ananassa Duch.). Proc. Amer. Soc. Hort. Sci. 90:153-157.

Eaks, I. 1985. Effect of calcium on ripening, respiratory rate, ethylene production, and quality of avocado fruit. J. Amer. Soc. Hort. Sci. 110:145-148.

Elkashif, M.E. 1985. Ethylene-induced tissue breakdown in watermelon (Citrullus lanatus (Thub.) Matsum and Kankai) fruit, pp. 43-55. PhD. dissertation, University of Florida.

Esau, K. 1977. Anatomy of Seed Plants, pp.442-450. J. Wiley and Sons, New York.

Ferreira, D.I. and G.H. DeSwardt. 1980. Effect of chemicals on respiration, vase-life and quality of cut flowers. Agroplantae 12:53-59.

Forney, C.F. and P.J. Breen. 1985a. Collection and Characterization of phloem exudate from strawberry pedicels. HortScience 20:413-414.

_____. 1985b. Growth of strawberry fruit and sugar uptake of fruit discs at different inflorescence positions. Sci. Hort. 27:55-62.

_____. 1986. Sugar content and uptake in the strawberry fruit. J. Amer. Soc. Hort. Sci. 111:241-247.

Fudge, B. 1930. Increasing the color of cranberries after removal from the vines. New Jersey Ag. Expt. Station Bull. 504.

Fuleki, T. 1969. The anthocyanins of strawberry, rhubarb, radish and onion. J. Food Sci. 34:365-369.

Gallender, J.F. 1979. Fruit concentration of amino acids in several strawberry cultivars in relation to the time of harvest. HortScience 14:48-49.

Goodenough, P.W. 1986. A review of the role of ethylene in biochemical control of ripening in tomato fruit. Plant Growth Regulation 4:125-137.

Gross, J. 1982. Changes of chlorophylls and carotenoids in developing strawberry fruits (Fragaria ananassa) cv Tenira. Gartenbauwissenschaft. 47:142-144.

Gross, K.C., A.E. Watada, M.S. Kang, S.D. Kim, K.S. Kim, S.W. Lee. 1986. Biochemical changes associated with the ripening of hot pepper fruit. Physiol. Plant. 66:31-36.

Gull, D.D. 1981. Utilization of the flow-through system for ripening initiation in tomatoes. Proc. Fla. State Hort. Soc. 94:289-291.

Guy, M. and H. Kende. 1984. Conversion of 1-amino-cyclopropane-1-carboxylic acid to ethylene by isolated vacuoles of Pisum sativum. Planta 160:281-287.

Hale, C.R., B.G. Coombe and J.S. Hauker. 1970. Effects of ethylene and 2-chloroethylphosphonic acid on the ripening of grapes. Plant Physiol. 45:620-623.

Halevy, A.H. and S. Mayak. 1979. Senescence and post-harvest physiology of cut flowers, Part 1. Hort. Rev. 1:204-236.

_____. 1981. Senescence and post-harvest physiology of cut flowers, Part 2. Hort. Rev. 3:59-143.

Havis, A.L. 1943. A developmental analysis of the strawberry fruit. Amer. J. Bot. 30:311-314.

Hirai, M. 1980. Sugar accumulation and development of loquat fruit. J. Japan. Soc. Hort. Sci. 49:347-353.

_____. 1982. Accelerated sugar accumulation and ripening of loquat fruit by exogenously applied ethylene. J. Japan. Soc. Hort. Sci. 51:159-164.

Ho, L.C. and R. Nichols. 1975. The role of phloem transport in the translocation of sucrose along the stem of carnation cut flowers. Ann. Bot. 39:439-446.

_____. 1977. Translocation of C-sucrose in relation to changes in carbohydrate content in rose corollas cut at different stages of development. Ann. Bot. 41:227-242.

Hobson, G.E., R. Nichols, J.N. Davies, and P.T. Atkey. 1984. The inhibition of tomato fruit ripening by silver. J. Plant Physiol. 116:21-29.

Hoffman, N.E. and S.F. Yang. 1980. Changes of 1-aminocyclopropane-1-carboxylic acid contents in ripening fruits in relation to their ethylene production rates. J. Amer. Soc. Hort. Sci. 105:492-495.

_____. 1982. Enhancement of wound-induced ethylene synthesis by ethylene in preclimacteric cantaloupe. Plant Physiol. 69:317-322.

Huber, D.J. 1984. Strawberry fruit softening: The potential roles of polyuronides and hemicelluloses. J. Food Sci. 49:1310-1315.

Huber, D.J. and M. Sherman. 1987. Preripening and ripening physiology, pp. 103-107. In: D.W. Newman, K.G. Wilson (Eds.), Models in Plant Physiology and Biochemistry, Vol. II. CRC Press, Boca Raton, FL.

Hyodo, H. and T. Nishino. 1981. Wound-induced ethylene formation in albedo tissue of citrus fruit. Plant Physiol. 67:421-423.

Imaseki, H. and A. Watanabe. 1978. Inhibition of ethylene production by osmotic shock. Plant Cell Physiol. 19:345-348.

Ismail, A.A. and W.J. Kender. 1969. Evidence of a respiratory climacteric in highbush and lowbush blueberry fruit. HortScience 4:342-344.

Janes, H.W., C.Chin and C. Frenkel. 1978. Respiratory upsurge in blueberries and strawberries as influenced by ethylene and acetaldehyde. Bot. Gaz. 139:50-52.

Janick, J. and D.A. Eggert. 1968. Factors affecting fruit size in the strawberry. Proc. Amer. Soc. Hort. Sci. 93:311-316.

Jeffery, D., C. Smith, P. Goodenough, I. Prosser and D. Grierson. 1984. Ethylene-independent and ethylene-dependent biochemical changes in ripening tomatoes. Plant Physiol. 74:32-38.

Kader, A.A. 1985. Ethylene-induced senescence and physiological disorders in harvested horticultural crops. HortScience 20:54-57.

Kader, A.A., R.F. Kasmire, F.G. Mitchell, M.S. Reid, N.F. Sommer and J.F. Thompson. 1985. Postharvest technology of horticultural crops, p.4. Univ. Calif. Div. Agriculture and Natural Resources. Davis, CA.

Kanellis, A.K., L.L. Morris, and M.E. Saltveit. 1986. Effect of stage of development on postharvest behavior of cucumber fruit. HortScience 21:1165-67.

Kano, Y. and T. Asahira. 1978. Effects of some plant growth regulators on the development of strawberry fruits in vitro culture. J. Japan. Soc. Hort. Sci. 47:195-202.

Kano, Y. and T. Asahira. 1981. Roles of cytokinin and abscisic acid in the maturing of strawberry fruits. J. Japan. Soc. Hort. Sci. 50:31-36.

Kende, H. and T. Boller. 1981. Wound ethylene and 1-amion-cyclopropane-1-carboxylate synthase in ripening tomato fruit. Planta 131:476-481.

Knee, M., J.A. Sargent and D.J. Osborne. 1977. Cell wall metabolism in developing strawberry fruits. J. Exp. Bot. 28:377-396.

Lam, P.F. and C.K. Wan. 1983. Climacteric nature of the caramboloa (Averrhoa carambola L.) Fruit. Pertanika 6:44-47.

_____. 1987. Ethylene and carbon dioxide production of starfruits (Averrhoa carambola L.) stored at various temperatures and in different gas and relative humidity atmospheres. Trop. Agric. 64:181-184.

Lee, J.S., J.Y. Pyon, and Y.R. Kim. 1980. Improving quality and prolonging vase life of cut flowers. J. Kor. Soc. Hort. Sci. 21:98-107.

Lieberman, M. 1979. Biosynthesis and action of ethylene. Ann. Rev. Plant Physiol. 30:533-591.

Lieberman, M., A.T. Kuneshi, L.W. Mapson, and D.A. Wardale. 1965. Ethylene production from methionine. Biochem. J. 97:449-459.

_____. 1966. Stimulation of ethylene production in apple tissue slices by methionine. Plant Physiol. 41:376-382.

Lis, E.K. and R. Antoszewski. 1979. Modification of the strawberry receptacle accumulation ability by growth regulators, pp. 263-270. In: R. Marcell, H. Clijster, M. Van Poucke (Eds.), Photosynthesis and Plant Development. Dr. W. Junk, Hague.

_____. 1982. Do growth substances regulate the phloem as well as the xylem transport of nutrients to the strawberry receptacle? Planta 156: 492-495.

Lis, E.K., B. Borkowska, R. Antoszewski. 1978. Growth regulators in the strawberry fruit. Fruit Sci. Reports 5:17-29.

Liu, Y., N.E. Hoffman, and S.F. Yang. 1985. Promotion by ethylene of the capability to convert 1-aminocyclopropane-1-carboxylic acid to ethylene in preclimacteric tomato and cantaloupe fruits. Plant Physiol. 77:407-411.

Lizada, M. C. and S.F. Yang. 1979. A simple and sensitive assay for 1-amioncyclopropane-1-carboxylic acid. Anal. Biochem. 100:140-145.

Loewus, F.A. and S. Kelly. 1961. The metabolism of D-galacturonic acid and its methyl ester in the detached ripening strawberry. Arch. Biochem and Biophys. 95:483-493.

Long, J. H. 1938. Seasonal changes in nitrogen and carbohydrate content of the strawberry plant. Proc. Amer. Soc. Hort. Sci. 36:495-497.

Lord, W.J. and D.G. White. 1962. The induction of parthenocarpy and an attempt to promote apomixis in the strawberry by treatments with growth regulators. Proc. Amer. Soc. Hort. Sci. 80:350-362.

Lurssen, K., K. Naumann and R. Schroder. 1979. 1-aminocyclopropane-1-carboxylic acid-an intermediate of the ethylene biosynthesis in higher plants. Z. Pflanzenphysiol. 92:285-294.

Marei, N. and J.C. Crane. 1971. Growth and respiratory response of Fig (Ficus carica L. cv. Mission) fruits to ethylene. Plant Physiol. 48:249-254.

Mason, D.T. and W.R. Jarvis. 1970. Postharvest ripening of strawberries. Hort. Res. 10:125-132.

Marousky, F.J. 1971. Inhibition of vascular blockage and increased moisture retention in cut roses induced by pH, 8-hydroxyquinoline citrate, and sucrose. J. Amer. Soc. Hort. Sci. 96:38-41.

Maxie, E.C., P.B. Catlin and H.T. Hartmann. 1960. Respiration and ripening of olive fruits. Proc. Amer. Soc. Hort. Sci. 75:275-291.

McGlasson, W.B. 1978. Role of hormones in ripening and senescence, pp. 77-96. In: H.O. Hultin and M. Milner, (eds) Postharvest Biology and Biotechnology. Food and Nutrition Press, Westport, CT.

_____. 1985. Ethylene and fruit ripening. HortScience 20:51-54.

McGlasson, W.B. and H.K. Pratt. 1964. Effects of ethylene on cantaloupe fruits harvested at various ages. Plant Physiol. 39:120-127.

McGlasson, W.B., H.C. Dostal and E.C. Tigchelaar. 1975. Comparison of propylene-induced responses of immature fruit of normal and rin mutant tomatoes. Plant Physiol. 55:218-222.

McMurchie, E.J., W.P. McGlasson, and I.L. Eaks. 1972. Treatment of fruit with propylene gives information about the biogenesis of ethylene. Nature 237:235-236.

Mitchell, F.G., E.C. Maxie and A.S. Greathead. 1964. Handling strawberries for fresh market. Div. Agricultural Science. Univ. Calif., Davis, CA. Circular 527, May-64.

Mizuno, S. and H.K. Pratt. 1973. Relation of respiration and ethylene production to maturity in watermelon. J. Amer. Soc. Hort. Sci. 98:614-617.

Moore, J.N., G.R. Brown and E.D. Brown. 1970. Comparison of factors influencing fruit size in large-fruited and small-fruited clones of strawberry. J. Amer. Soc. Hort. Sci. 95:827-831.

Mudge, K. W., K.R. Narayanan, and B.W. Pooviah. 1981. Control of strawberry fruit set and development with auxins. J. Amer Soc. Hort. Sci. 106:80-84.

Nakajima, N. and H. Imaseki. 1986. Purification and properties of 1-aminocyclopropane-1-carboxylate synthase

of mesocarp of Cucurbita maxima Duch. fruits. Plant Cell Physiol. 27:969-980.

Neal, G.E. 1965. Changes occurring in the cell walls of strawberries during ripening. J. Sci. Fd. Agric. 16:604-611.

Nitsch, J.P. 1950. Growth and morphogenesis of the strawberry as related to auxin. Amer. J. Bot. 37:211-215.

_____. 1951. Growth and development in vitro of excised ovaries. Amer. J. Bot. 38:566-577.

Olsen, J.L., L.W. Martin and P.J. Breen. 1985. Yield component analysis of 'Benton' and 4356 strawberries. HortScience 20:74-76.

Oslund, C.R. and T.L. Davenport. 1983. Ethylene and carbon dioxide in ripening fruit of Averrhoa carambola. HortScience 18:229-230.

Paulin, A. 1986. Influence of exogenous sugars on the evolution of some senescence parameters of petals. Acta Hort. 181:183-193.

Paull, R.E. 1982. Postharvest variation in composition of Soursop (Annona murcata L.) fruit in relation to respiration and ethylene production. J. Amer. Soc. Hort. Sci. 107:582-585.

Pratt, H.K., J.D. Goeschl and F.W. Martin. 1977. Fruit growth and development, ripening and the role of ethylene in the 'Honeydew' muskmelon. J. Amer. Soc. Hort. Sci. 102:203-210.

Privalle, L.S. and J.S. Graham. 1987. Radiolabelling of a wound-inducible pyridoxal phosphate-utilizing enzyme: evidence for its identification as ACC synthase. Arch. Biochem. Bioph. 253:333-340.

Reid, M.S., D.S. Farnham and E.P. McEnroe. 1980a. Effect of silver thiosulfate and preservative solutions on the vase life of miniature carnations. HortScience 15:807-808.

Reid, M.S., J.L. Paul, M.B. Farhoomand, A.M. Kofranek, and G.L. Staby. 1980b. Pulse treatments with the silver thiosulfate complex extend the vase life of cut carnations. J. Amer. Soc. Hort. Sci. 105:25-27.

Reid, M.S., J.C. Pech and A. Latche. 1985. Non-intervention de l'ethylene endogene dans la

maturation de la cerise, fruit non-climacterique. Fruits 40:197-203.

Reyes, F.G., R.E. Wrolstead and C.J. Cornwell. 1982. Comparison of enzymic, gas-liquid chromatographic, and high performance liquid chromatographich methods for determining sugars and organic acids in strawberries at three stages of maturity. J. Assoc. Off. Anal. Chem. 65:126-131.

Rhodes, M.J.C. 1980. The maturation and ripening of fruits, pp. 157-205. In: K. Thimann (Ed.), Senescence in Plants. CRC Press, Boca Raton, FL.

Riov, J. and S.F. Yang. 1982a. Autoinhibition of ethylene production in citrus peel discs. Plant Physiol. 69:687-690.

_____. 1982b. Effect of cycloheximide on ethylene production in intact and excised citrus fruit tissues. J. Plant Growth Regul. 1:95-104.

Ryan, J.J. 1971. The anthocyanins of strawberry, rhubarb, radish and onion. J. Food Sci. 34:365-369.

Saftner, R.A. 1986. Effect of ethylene on sucrose uptake in root discs of sugar beet. Plant Cell Physiol. 27:853-860.

Saltveit, M.E. 1977. Carbon dioxide, ethylene, and color development in ripening mature green bell peppers. J. Amer. Soc. Hort. Sci. 102:523-525.

Saltveit, M.E., K.J. Bradford, D.R. Dilley. 1978. Silver ions inhibits ethylene synthesis and action in ripening fruits. J. Amer. Soc. Hort. Sci. 103:472-475.

Sherman, W.B. and J. Janick. 1966. Greenhouse evaluation of fruit size and maturity in strawberry. Proc. Amer. Soc. Hort. Sci. 89:303-308.

Shimura, I., M. Koayashi, and S. Ishikawa. 1986. Characteristics of fruit growth and development in high-bush and rabbiteye blueberryies (*Vaccinium corymbosum* L. and *V. ashei* Reade) and the differences among their cultivars. J. Japan. Soc. Hort. Sci. 55:46-50.

Shoemaker, J.S. 1978. Strawberries, pp. 103. In: J.S. Shoemaker (Ed.), Small Fruit Culture. AVI Publishing Co., Westport, CT.

Sitrit, Y., J. Riov, and A. Blumenfield. 1986. Regulation of ethylene biosynthesis in avocado fruit during ripening. Plant Physiol. 81:130-135.

Smith, W.L. and P.H. Heinze. 1958. Effect of color development at harvest on quality of post-harvest ripened strawberries. Proc. Amer. Soc. Hort. Sci. 72:207-211.

Stutte, G.W. and R.L. Darnell. 1987. A nondestructive developmental index for strawberry. HortScience 22:218-220.

Terai, H. and S. Mizuno. 1985. Changes of 1-aminocyclopropane-1-carboxylic acid (ACC) and ethylene forming enzyme activity in growing and ripening fruits of tomato and cucumber. J. Japan. Soc. Hort. Sci. 53:467-473.

Thompson, P.A. 1963. The development of embryo, endosperm, and nucellus tissues in relation to receptacle growth in the strawberry. Ann. Bot. 27:589-605.

_____. 1964. The effect of applied growth substances on development of the strawberry fruit. J. Exp. Bot. 15:347-358.

_____. 1969. The effect of applied growth substances on development of strawberry fruit. II. Interactions of auxins and gibberellins. J. Exp. Bot. 20:629-647.

Tingwa, P.O. and R.E. Young. 1975. Studies on the inhibition of ripening in attached avocado (Persea americana Mill.) fruits. J. Amer. Soc. Hort. Sci. 100:447-449.

Van Meeteren, U. 1978. Water relations and keeping-quality of cut gerbera flowers. I. the cause of stem break. Sci. Hort. 8:65-74.

Veen, H. 1983. Silver thiosulphate: an experimental tool in plant science. Sci. Hort. 20:211-224.

_____. 1985. Antagonistic effect of silver thiosulphate or 2,5-norbornadiene on 1-amioncyclopropane-1-carboxylic acid-stimulated growth of pistils in carnation buds. Physiol. Plant. 65:2-8.

_____. 1986. A theoretical model for anti-ethylene effects of silver thiosulphate and 2,5-norbornadiene. Acta Hort. 181:129-134.

Veluthambi, K. and B.W. Pooviah. 1984. Auxin-regulated polypeptide changes at different stages of strawberry fruit development. Plant Physiol. 75:349-353.

Vines, H.M., G.J. Edwards and W. Grierson. 1965. Citrus fruit respiration. Proc. Fla. State. Hort. Soc. 78:198-202.

Wang, C.Y. and D.O. Adams. 1982. Chilling-induced ethylene production in cucumbers (Cucumis sativus L.). Plant Physiol. 69:424-427.

Watada, A.E., R.C. Herner, A.A. Kader, R.J. Romani, and G.L. Staby. 1984. Terminology for the description of developmental stages of horticultural crops. HortScience 19:20-21.

Webb, R.A., J.H. Terblanche, J.V. Purves, and M.G. Beech. 1978. Size factors in strawberry fruit. Sci. Hort. 9:347-356.

Woodward, J.R. 1972. Physical and chemical changes in developing strawberry fruits. J. Sci. Food Agric. 23:465-473.

Wrolstad, R.E. and R.S. Shallenberger. 1981. Free sugars and sorbitol in fruits--a compilation from the literature. J. Assoc. Off. Anal. Chem. 64:91-103.

Yang, S.F. 1980. Regulation of ethylene biosynthesis. HortScience 15:238-243.

_____. 1981. Biosynthesis of ethylene and its regulation, pp.89-106. In: J. Friend, M.J.C. Rhodes, (Eds.), Recent Advances in the Biochemistry of Fruit and Vegetables. Academic Press, London.

_____. 1985. Biosynthesis and action of ethylene. HortScience 20:41-45.

_____. 1987. The role of ethylene and ethylene synthesis in fruit ripening, pp.156-166. In: K. Thimann (Ed.), Plant Senescene. CRC Press, Boca Raton, FL.

Yang, S.F. and N.E. Hoffman. 1984. Ethylene biosynthesis and its regulation in higher plants. Ann. Rev. Plant Physiol. 35:155-189.

Yang, S.F., Y. Liu, and O.1. Lau. 1986. Regulation of ethylene biosynthesis in ripening apple fruit. Acta Hort. 179:711-720.

Yen, H.F. and L. Tzong-shyan. 1986. Respiratory pattern and autocatalytic ethylene production of two cultivars of Psidium guajava L. J. Chinese Soc. Hort. Sci. 32:224-232.

Yu, Y.B. and S.F. Yang. 1979. Auxin-induced ethylene production and its inhibition by aminoethoxyvinylglycine and cobalt ion. Plant Physiol 64:1074-1077.

Yu, Y.B., D.O. Adams, and S.F. Yang. 1979. 1-aminocyclopropanecarboxylate synthase, a key enzyme in ethylene biosynthesis. Arch. Biochem. Biophys. 198:280-286.

Zagory, D. and M.S. Reid. 1986. Role of vase solution microorganisms in the life of cut flowers. J. Amer. Soc. Hort. Sci. 111:154-158.

Zauberman, G. and Y. Fuchs. 1973. Ripening processes in avocados stored in ethylene atmosphere in cold storage. J. Amer. Soc. Hort. 98:477-480.

Zieslin, N., H.C. Kohl, A.M Kofranek and A.H. Halevy. 1978. Changes in the water status of cut roses and its relationship to bent-neck phenomenon. J. Amer. Soc. Hort. Sci. 103:176-179.

BIOGRAPHICAL SKETCH

Penelope Marie Perkins-Veazie was born in Auburn, Maine, on December 18, 1958, to Sandra and Lewis Perkins. She was raised in Buckfield, a Maine village in the foothills of the White Mountains. Following graduation in 1977 from Buckfield High School, she enrolled at the University of Maine at Orono. She was awarded a B.S. degree in plant and soil sciences in 1981. She moved to Gainesville, Florida, in 1981 and enrolled at the University of Florida. She completed her Master of Science degree in 1985.

The author has been married to Raymond J. Veazie since 1980. The author has accepted a postion as a postharvest physiologist with the United States Department of Agriculture and will be stationed at the Wes Watkins Research Facility in Lane, Oklahoma.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Donald J. Huber, Chair
Associate Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Jeffrey K. Brecht, Co-Chair
Assistant Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Earl E. Albregts
Professor of Soil Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Thomas E. Humphreys
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Chesley B. Hall
Professor of Horticultural Science

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

April 1988

Jack L. Fry
Dean, College of Agriculture

Dean, Graduate School